

Tracts 1373

(1.)

With the author's
kind regards

ON THE PHYSIOLOGY OF THE
EMBRYONIC HEART,

BEING A

THESIS FOR THE DEGREE OF DOCTOR OF SCIENCE
OF THE UNIVERSITY OF LONDON, 1893,

BY

J. W. PICKERING, D.Sc.



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Dr. Lucy Power Esq. matres. &c.
With the Author's Compliments

ON THE PHYSIOLOGY OF THE EMBRYONIC HEART
(PRELIMINARY COMMUNICATION).

BY

J. W. PICKERING, B.Sc.



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“On the Physiology of the Embryonic Heart (Preliminary Communication).” By J. W. PICKERING, B.Sc., Assistant Demonstrator in Biology at St. Bartholomew’s Medical School. Communicated by Professor HALLIBURTON, F.R.S. Received November 25, 1892.

(From the Physiological Laboratory of King’s College, London.)

The object of the following experiments has been to study the effect of varying conditions on the heart previous to the development of a nervous mechanism, and thus to throw some light on the discussion as to the relative importance of the two factors in the heart’s action, viz., the contractile tissue and the nervous elements. The heart I have used is that of the chick* at a period of incubation of seventy-two hours at a temperature of 38° C. In some cases the embryos have been a few hours older or younger. The embryo is not removed from the egg, but a window is cut 3 em. square through the shell and shell membrane, exposing the albumen and blastoderm, which remain undisturbed; the egg and embryo is fixed in a small chamber surrounded on five sides by a water-jacket. The uppermost side is covered with glass, while the air of the chamber is kept moist by the evaporation of water from a small bowl placed inside it. The temperature of this chamber can be kept constant or varied at pleasure. My experiments have fallen under three main heads: 1. The results of varying the temperature. 2. The introduction of drugs. 3. Electrical stimulation. In my full paper the results will be shown in tables giving the number of heart beats per minute, the peculiarities in the beat, when such exist, being duly noted. At present, however, I am only prepared to give an abstract of the results obtained, in so far as temperature and drugs are concerned. The electrical experiments are not yet completed.

* Observations are being carried on upon the mammalian embryo *in situ*

1. *Temperature.*

Each embryo has an individual rhythm of its own, which, if the conditions are constant, remains unaltered, but different embryos, even of the same age, may have different rhythms, so that it is necessary to determine for each embryo its normal rhythm before variations can be studied. An embryo's heart, aged seventy-two hours, at a temperature of 31° C., was beating with a regular rhythm of 84 per minute. The temperature of the air of the chamber was rapidly raised to 42° C., when the rhythm rose to 91 per minute. A further rise to 50° C. increased the rhythm to 128, it still remaining regular. The temperature was then rapidly lowered to 26° C., when the rhythm fell to 114 per minute. A further fall to 16° C. reduced the rhythm to 34 per minute. The temperature was then raised to 46° C., when the rhythm rose to 117° per minute. On again letting the temperature fall to 25° C., the rhythm fell to 36° per minute.

The above experiment, taken as an instance from several, shows that, other factors being constant, the rhythm of the embryonic heart varies directly with the temperature of the surrounding medium.

Extremes of temperature stop the heart; thus exposure to a temperature of 10° C. causes the beats to become weaker and slower, and finally to stop in diastole. If the air of the incubator be raised above 50° C., the beats become so rapid as to be uncountable. They are feeble, and the heart is pale, due to the passage of less blood through it than in the normal state. Violent systolic spasms alternate with periods of quiescence. It stops in an expanded condition when the surrounding temperature is about 55° C. Lowering the temperature restores the beating, but the heart is enfeebled. If the temperature is raised much above this limit the heart is killed. Mechanical stimulation of the heart in standstill, due to either extreme of temperature, if applied at the ventricular end, gives rise to one or more waves of contraction, commencing from the auricular end, and showing the direct conduction through the fibres of the heart. The heart will respond to auricular stimulation when irresponsible to ventricular stimulation. Small variations of temperature, such as one or two degrees, occurring over a long period of time, as in an hour, do not affect the rhythm.

2. *The Introduction of Drugs.*

The drugs employed were applied directly to the heart substance at the temperature of the embryo, and dissolved in normal saline (0.65 per cent. sodium chloride) solution.

a. *Caffeine.*—An embryo, aged sixty-eight hours, at 33° C. had a rhythm of 88 per minute. To its heart 0.00015 gram* of caffeine was

* All weights of drugs used are expressed in grams.

administered, and in two minutes the rhythm rose to 100 per minute, and remained constant for two and a half minutes, when it fell to 96 per minute. A second dose of 0.00015 gram raised the rate to 102 per minute. The beats were also of greater force, since more blood was seen passing through the heart. A dose of 0.0025 gram was fatal. When given to an embryo, aged seventy-five hours, at 37° C., beating with a rhythm of 116 per minute, it reduced the rhythm, after one minute's action, to 100 per minute. The beats, however, remained very strong. After one minute forty-five seconds' action the heart stopped in strong systole, but started again and gave a few powerful beats. After the drug had acted nine minutes thirty seconds the heart stopped permanently in powerful contraction. Caffeine, therefore, acts directly on the cells of the embryonic heart.

b. *Strychnine* was given to a seventy hours' embryo in a dose of 0.000017 gram, and depressed the rhythm of the heart from 112 per minute to 52 per minute. There was no spasm. In an eighty hour embryo, at 39° C., a dose of 0.00002 gram temporarily increased the rhythm, both in force and number of beats; then the systole rapidly became weakened and the rhythm irregular. A further dose of 0.00002 gram still more rapidly reduced both force and frequency of beating, till death in diastole occurred.

c. *Morphine acetate*, if given in doses of 0.0001 gram, is a powerful depressant. With a dose of 0.0002 gram, after one minute's action on an eighty-five hours' embryo at 40° C., irregularities and slowing were obtained; after two minutes' action the beating stopped, but went on again, the waves of contraction sometimes passing from ventricle to auricle, and at others in the normal direction. Periods of rest alternated with violent bouts of rapid beating.

d. *Veratrine*.—Doses of 0.0001 gram increase the number of beats per minute. Larger doses may cause, temporarily, an increase of rhythm, but soon depress the heart by greatly lengthening the systole, which becomes very weak while the diastole is complete. The heart stops in an expanded condition. The heart of a seventy-two hours' embryo that had stopped in diastole, after a dose of 0.0005 gram, was restored by the application of 0.01 gram of potassium chloride almost to its normal rhythm. This agrees with Ringer's observation on the frog's heart.

e. *Potassium chloride*, when applied in a dose of 0.005 gram to an embryo aged seventy-two hours, reduced the normal rhythm of 76 per minute to 60 per minute. A further dose of 0.01 gram reduced the rhythm to 64 per minute. After the administration of a total amount of 0.07 gram of the substance, the heart stopped in diastole.

f. *Nicotine*, in very minute doses, stimulated the embryonic heart; $\frac{1}{4}$ c.c. of a solution containing $\frac{1}{2}$ c.c. of nicotine to 100 c.c. of normal

saline was a stimulant; with $\frac{1}{2}$ c.c. the frequency and force of the heart diminished, systole becoming almost absent, while the heart was finally paralysed in diastole. The addition of 0.03 gram of potassium chloride restored the heart to almost its normal rhythm, the beats at the same time becoming strong, both as regards systole and diastole. A further dose of nicotine depressed the heart, and again brought it into diastolic stoppage, the systoles having become weaker and weaker. There was no spasm.

g. *Atropine*.—Doses of 0.001 gram had, in a sixty hours' embryo, a slightly depressant effect, and even after 0.006 gram had been administered, the rhythm of the heart had only fallen from 96 to 72 per minute. In a seventy-two hours' embryo, with a heart beating at 116 per minute, 0.012 gram, after three minutes' action, had depressed the rhythm to 80 per minute, while even after the administration of 0.275 gram the rhythm was strongly maintained at 64 per minute.

h. *Muscarine Nitrate*.—To the heart of a seventy-two hours' embryo at 35° C., which was beating with a rhythm of 90 per minute, 3 drops of half saturated solution of muscarine nitrate were applied; the rhythm remained constant for two minutes, after which period 2 more drops were added, and the rhythm kept constant at 94 per minute during the next three minutes, after which period 4 more drops were added, and the ensuing rhythm was 93 per minute; 2 drops of saturated solution were then added, which was so concentrated as to stain the embryo brown. During the following five minutes the rhythm was constant at 84 per minute, each beat remaining normal in direction and force. Two more drops of saturated solution caused slight irregularities, but the rhythm during the next seven minutes averaged 72 beats per minute. Finally 2 more drops of saturated solution were added, and during the following seven minutes the heart's rhythm was 75 per minute. The whole experiment lasted thirty minutes, and 10 drops of half saturated *plus* 9 drops of saturated solution of muscarine nitrate were administered. A control experiment with the hearts of two frogs showed that the muscarine used stopped their beats, which were typically restored by atropine. In a similar experiment, witnessed by Professor Halliburton, with both embryonic and frogs' hearts, the rhythm of the former was maintained at 136 per minute, while the latter was stopped and subsequently restored by atropine. Identical results were obtained with a ninety-six hours' embryo. In an embryo aged seventy hours at a temperature of 30° C., which is subnormal in the chick, a rhythm of 92 beats was obtained after the application of 1 c.c. of half saturated solution for the following nine minutes, after which 1 c.c. of saturated solution was applied. This was fatal to the heart, almost instantly coagulating the tissues. There were no typical phenomena

of muscarine poisoning, and the application of atropine failed to restore the rhythm. Probably any strongly alkaloidal body in such a concentrated solution would produce a similar effect.

i. *Schmiedeberg's Digitalin*.—An embryo aged seventy-two hours at 30° C. had a heart rhythm of 132 per minute. To it 1 c.c. of normal saline containing 0.000022 gram of digitalin was applied. During the next eleven minutes the rhythm remained constant, after which time 1 c.c. containing 0.00005 gram was added, which produced no change in the rhythm; then 0.0001 gram was put in, and after one minute's action the frequency of the rhythm had fallen to 92 per minute, but both the systole and diastole were strong. The rhythm after six minutes' action rose to 104 per minute. After this another 0.0001 gram was added, and the rhythm fell after two minutes' action to 50 per minute. The systole was typically perfect, but the diastole was incomplete. The whole heart after two minutes' more action of the drug became very pale and in a state of tonic contraction with very feeble fluttering diastoles, which faded away, leaving the heart stopped in a contracted condition.

j. *Strophanthin* (of Merck's manufacture).—A seventy-two hours' embryo at a temperature of 32° C. had a heart rhythm of 132 per minute. A dose of 0.00006 gram did not alter the rhythm. A second dose of the same amount after twenty minutes' action reduced the rhythm to 54 per minute; both systole and diastole were regular and complete. Five minutes after this the diastole became irregular, and the systole was more marked than in the normal condition. After another minute had elapsed the ventricle passed into a state of tonic contraction with a few feeble beats, in which the diastole was very weak. The auricles had a marked diastole and a weak systole, and were engorged with blood. During the next five minutes the auricle had a rhythm averaging 24 beats per minute, while the ventricle remained in tonic contraction. Finally, forty-one minutes after the administration of the dose the auricle stopped in diastole, the ventricle remaining in tonic contraction. The auricles responded by 10 beats to a mechanical stimulus; the beats did not extend to the ventricle. Six minutes after this the auricle responded to mechanical stimuli, the wave of contraction passing either from the ventricular end to the auricle or *vice versa*, according to which end of the auricle the stimulus was applied.

In larger doses of 0.0002 gram the rhythm in a seventy hour embryo at 33° C. was depressed from 120 to 102 per minute, the systole becoming very strong and the diastole imperfect. After four minutes' action the rhythm returned to the normal both in frequency and force. To the same embryo 0.00025 gram was then added, when after one minute's action the auricle dilated, giving small twitch-like contractions, while the ventricle passed into tonic contraction. The

auricle remained for six minutes feebly responsive to mechanical stimuli.

k. *Nitrite of Amyl.*—A ninety-six hours' embryo kept at 35° C. was subjected to the influence of the vapour of 5 minims of nitrite of amył. After one minute's action the rhythm rose from 96 to 124, and after another minute fell to 112. After another minute it had fallen to 104, and six minutes afterwards was at the normal. In a seventy-two hour embryo at a temperature of 47° the rhythm was 124 per minute. A dose of 1 c.c. of solution of amył nitrite dissolved in olive oil (strength being 1·5 c.c. of the drug to 10 c.c. of olive oil) was given, and the frequency of the rhythm fell in one minute to 112, but the beats were strong. Six minutes afterwards another c.c. of the solution was introduced, and the rhythm fell to 104, but was strong. Three minutes later another c.c. was put in, and the rhythm rose to 112, but was very weak and irregular, and finally before death the rhythm was reversed.

Concluding Remarks.

The observations here recorded show that the embryonic heart when kept under favourable conditions reacts in a very delicate manner to all those classes of stimuli which influence the adult heart. The experiments on temperature show that its variations act directly on the cardiac muscle, and thus confirm the opinion of Newell Martin* and others who have arrived at the same conclusion from experiments on the adult heart.

The action of caffeine, morphine acetate, potassium chloride, veratrine, nicotine, digitalin, strophanthin, and amył nitrite is direct on the contractile tissue of the embryonic heart. This greatly favours the view that they act direct on the adult cardiac muscle. It will be noted that many of the actions here described on the embryonic heart are almost identical to those observed by others on the adult heart. Notoriously so is the antagonism between veratrine and potassium chloride, where my observations are identical with those of Ringer† on the frog's heart. A similar antagonism exists between nicotine and potassium chloride. The remarkable correspondence of my results with strophanthin on the embryonic heart with those of Professor Fraser‡ on the frog's heart greatly supports the view of that observer as to the direct action of strophanthin on cardiac muscle without the intervention of any nervous mechanism, and, further, the absence of diastolic stoppages in my experiments also supports Fraser's view that that condition in the frog's heart is due to the

* Newell Martin, 'Phil. Trans.,' 1883, p. 663.

† Ringer, 'Practitioner,' vol. 30 (1883), p. 17.

‡ Fraser, 'Edinburgh Roy. Soc. Trans.,' vol. 36 (1890-91), Part II, p. 388, *et seq.*

action of small doses of strophanthin on the cardiae nervous mechanism of that animal.

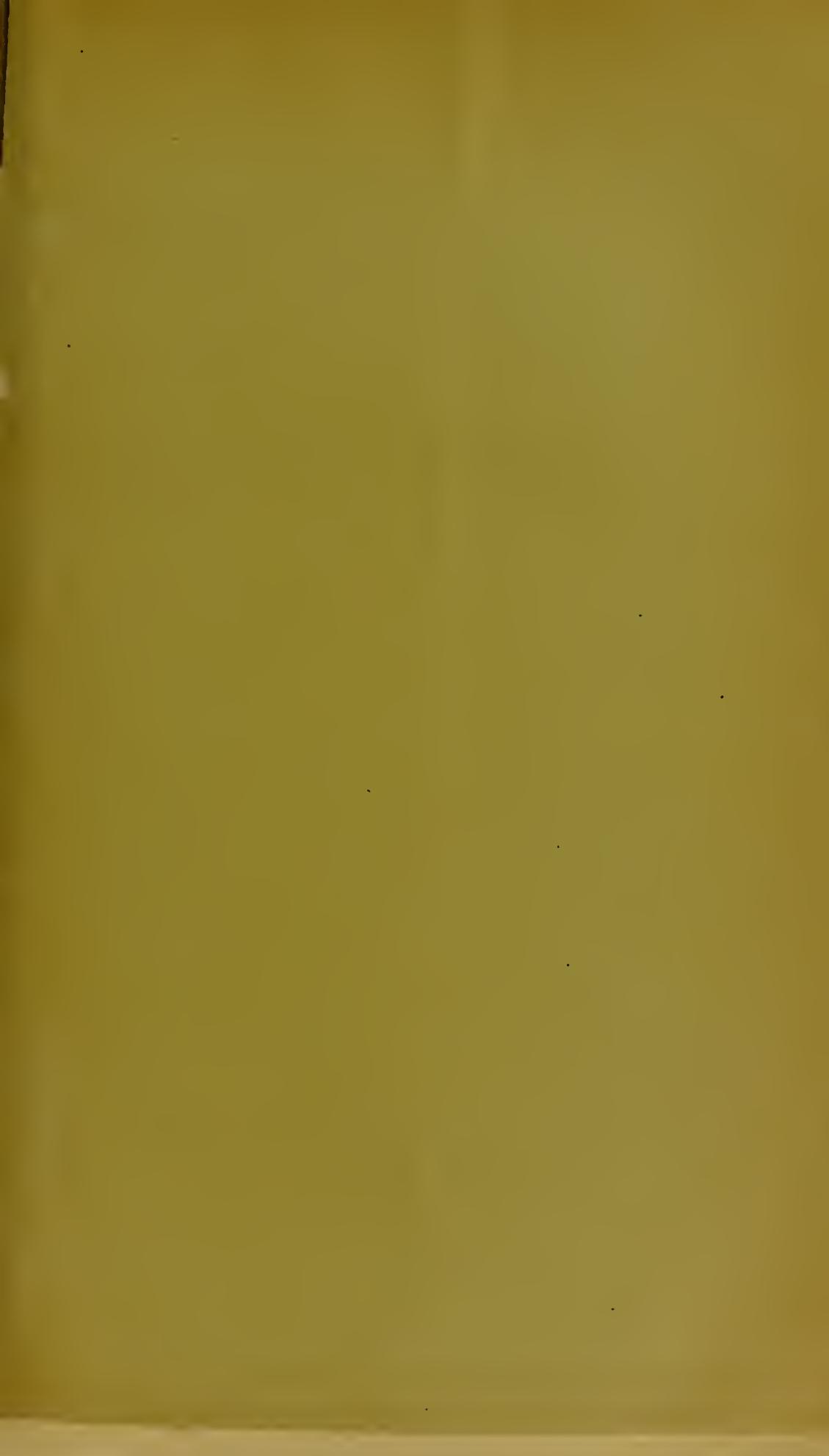
The lengthening out of the systole in veratrine poisoning corresponds to the same well-known lengthening of the systole in the frog's heart under veratrine. The reversing of rhythm observed in morphine poisoning is similar to that mentioned by Ludwig* as occurring in the mammalian ventricle when under the influence of opium, for then the auricular beats follow instead of precede the ventricular beats, the rhythm being reversed. The same occurs in amyl nitrite poisoning.

Krukenberg† has stated that neither atropine nor muscarine affects the heart of Ascidiants.

My observations on the action of atropine and muscarine, which have been made on a large number of embryos, show that in the absence of a nervous mechanism they do not influence the heart. This will probably modify the current views on the action of these drugs, and my results show that the method I have adopted is a valuable one for differentiating the functions of cardiac muscle from those of the nerves which supply it.

* Ludwig, 'Lehrbuch der Physiol. des Menschen,' Bd. 2 (1861), p. 38.

† Krukenberg, quoted in Brunton's 'Text-Book of Pharmacology,' &c. (3rd edition, p. 114).





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OBSERVATIONS ON THE PHYSIOLOGY OF THE EMBRYONIC HEART. BY JOHN W. PICKERING, B.Sc. (Lond.), Assistant Demonstrator in Biology at St Bartholomew's Medical School. (Plate XXIV.)

(From the *Physiological Laboratory of King's College, London.*)

THE attention of physiologists has for many years been directed to the discussion of the relative importance of the nervous and muscular mechanisms which control the rhythm and automatism of the heart. The classical researches of Stannius¹, Harnack², and Schmiedeberg³ emphasised the importance of the nervous elements, while Merunowicz⁴, Bowditch⁵, Foster⁶, Roy⁷ and Gaskell⁸ have advanced our knowledge of the muscular system. In a previous paper⁹ I have pointed out that the embryonic heart of the chick between the ages of 60 and 75 hours, owing to the absence of nervous elements, presents us with a convenient method of differentiating the functions of cardiac contractile tissue from the nerves which supply it.

The most important experiments on the same line of research are those of Gaskell, who worked with the "apex" of batrachian and chelonia hearts, since ganglia are absent from this portion of the heart. In considering the results of my observations in the relation to the experiments on the apex of the heart it must not be forgotten that the apex

¹ Stannius. *Müller's Archiv*, 1852. S. 95.

² Harnack. *Die Bedeutung pharmakologischer Thatsachen für die Physiologie des roschherzens*. Halle, 1881.

³ Schmiedeberg*. *Ludwig's Arbeiten*, 1870. S. 41.

⁴ Bowditch*. *Ludwig's Arbeiten*. Bd. vi. (1872), S. 139.

⁵ Merunowicz*. *Ludwig's Arbeiten*. Bd. (1875), S. 132.

⁶ Foster*. *Pflüger's Archiv*. Bd. v. S. 191. *Proc. Roy. Soc.* Vol. xxiii. p. 318.

⁷ Roy*. *This Journal*. Vol. i. p. 496.

⁸ Gaskell*. *This Journal*. Vol. iii. p. 108.

⁹ *Proc. Roy. Soc.* Vol. lii. (1893), p. 461.

* Other papers cited in the body of the paper.

is the least differentiated portion of that organ, and that as Roy has pointed out it is the auricle that regulates the work of the heart or as Wesley-Mills¹ aptly expresses it, "The tendency to spontaneous contraction of the muscle is most marked in the oldest part of the heart considered ancestrally." As I hope to shew in the following pages that there are considerable differences in the attributes of the auricular contractile tissue and the ventricular contractile tissue in the three-day chick embryo, it is important to bear in mind that in my experiments the whole heart is under observation. Newell-Martin² in discussing the action of heat upon the dog's heart mentions Cleland's observation on the chick's heart, and in criticising it, remarks that the embryonic heart on account of its protoplasmic nature might differ markedly from the adult heart. It must however be borne in mind that the fibres of the adult heart are in many respects primitive, as is shewn by their histological structure. It will be subsequently pointed out that in response to all classes of stimuli the embryonic heart shows striking similarity to the adult heart, especially in its response to the application of certain drugs (*e.g.* digitalin, and strophanthin) whose action has been assigned to be upon the cardiac muscle itself. Further, that where divergences occur between the action of certain drugs on the embryonic heart, and their action upon the adult heart, these differences are in many cases explainable by the absence of a nervous mechanism. The contractile substance of the embryonic heart at the age at which these experiments were conducted I have denoted by the term myoplasm. My experiments have fallen under two main heads,

- (1) The results of varying the temperature.
- (2) The introduction of drugs.

I have laid particular stress upon the action of chemical stimuli, because I believe that this line of work coupled with the advancing knowledge of the chemistry of protoplasm must lead to a more intimate knowledge of contractile tissues, while the connection between chemical constitution and physiological action should throw some light on the molecular changes which contractile tissues undergo when acted upon by drugs. I have also given some prominence in discussing my results to their relation to the observations of other observers on various vertebrate and invertebrate types, as the comparative method is less

¹ Wesley-Mills*. *Canada Medical and Surgical Journal*, 1887, p. 321—30.

² Newell-Martin*. *Phil. Trans. Roy. Soc.* 1883.

* Other papers cited in the body of the paper.

liable to lead to error than the results obtained from the study of a single class of animals.

Historical Summary of previous work on the Embryonic Heart.

The phenomena of the embryonic heart have been observed since the earliest times, since Aristotle⁽¹⁾ mentions the “στιγμὴ κινουμένη” in the hatching hen’s egg. Hieronymus Fabricius⁽²⁾ figured in his treatise on the incubation of the hen’s egg, the *arca vasculosa*. Harvey⁽³⁾ wrote of the embryonic heart “about the end of the fourth and the beginning of the fifth day, being now enlarged, it seemeth to be changed into a small thin bladder containing blood which it ejects at every contraction and recalls at every diastole.” He further found that touching the heart either caused it to beat faster or stopped it altogether, a fact which has been recently confirmed by Sonnenkalb. He also observed that cold delays and finally stops its beating and that on warming it “presently gaineth strength and vigour.”

Langley⁽⁴⁾ described two moving points at the centre of the vascular area of the embryo. This was confirmed by Maître Jean⁽⁵⁾ and by Lancisi⁽⁶⁾, who worked under Malpighi’s direction.

Vieussens⁽⁷⁾ pointed out what previous observers had (excepting Harvey) not noticed, viz., the morphological identity of the moving embryonic points with the adult heart. He also laid stress upon the importance of the elasticity of the cardiac muscular fibres in considering the causes of the rhythm of the heart, a factor which has recently been brought into notice by the researches of Roy⁽⁸⁾.

The subject was more fully investigated by Haller⁽⁹⁾, who used the evidence of the embryonic heart in support of his famous doctrine that the rhythmic contraction of the cardiac fibres depends on their inherent irritability. To those who stated that the systolic phase of the heart cycle was due to compression of the nerves, he answered “in fish, and in little chicklings in the egg there can be no room for a compression of the nerves.” He first observed the embryonic heart beat at 45 hours. Von Baer⁽¹⁰⁾ and Remak⁽¹¹⁾ observed it at about the same period of incubation, while Prévost and Dumas⁽¹²⁾ saw it at the 36th or 39th hour.

Von Baer observed that the first contractions of the embryonic heart were irregular, but this was thought by His⁽¹³⁾ to be due to the younger embryos being more influenced by cold than those more mature.

The more recent work of Wernicke and of Preyer⁽¹⁴⁾ has shewn that the first heart-beats are irregular, with long pauses between each bout of beating like those observed by Luciani⁽¹⁵⁾ in frog's hearts under certain conditions.

The normal rhythm of the chick's heart has been investigated by several observers. Remak speaks of 40 systoles per minute. Von Baer of 150 per minute, while Köllicker⁽¹⁶⁾ places the heart frequency of young embryos from 40 to 60 beats per minute. Wernicke⁽¹⁷⁾ who experimented upon three-day embryos found the frequency of their heart rhythm, taken during the thirty seconds which immediately follow the opening of the egg, to vary from 90 to 146 beats per minute in the ten observations he made. Similar observations were made up to the fifth day of incubation, shewing variations of frequency as much as 58 beats per minute for embryos of the same age. It is however evident from Wernicke's table that the older embryos have in general a greater frequency than those of an earlier date. Preyer⁽¹⁸⁾ found similar variations between the 46th and 170th hour.

Eckhard⁽¹⁹⁾ found that in 8 to 10 day chick embryos the heart-beats were arrested by a temperature of 41° or 42° C. He thought the heat acted directly upon the cardiac muscular fibres and not through the intermediary of any nervous mechanism as was the current view of the action of heat on the heart at this period. He also separated the ventricle from the auricle and found the ventricle then stopped in diastole. It however recommenced beating when heated, and stopped beating when cooled.

Schenk⁽²⁰⁾, who experimented with three-day embryos, found that an increase of temperature alone caused an increase of the frequency of the heart rhythm, and that the heart stopped when the temperature was either raised above 40° C. or fell below 23° C. A heart that had been exposed to a temperature of 8° C. recommenced beating when it was heated to 34° C. Wernicke and Preyer found that the embryonic heart reacted very delicately to variations of temperature. Below 10° C. the heart stopped in diastole but on raising the temperature it recommenced beating. A rise to 43° C. increased the frequency of the rhythm until it became uncountable. At 50° C. it ceased altogether.

Cleland⁽²¹⁾ observed the excised blastoderm of an embryo that had been hatched for 52 hours. He found the heart beating with a frequency of 36 per minute. On exposing the embryo to the cold of the surrounding atmosphere its heart stopped beating, but by bringing it near to a lighted lamp he increased the rhythm to 100 per minute.

He concludes his observation thus, "It is interesting to note that in the chick, where one homogeneous structure apparently combines the rhythmic impulse, excitability by artificial irritation and contractility, the effects of heat are the same as on the fully formed heart." Darest⁽²²⁾, who obtained similar results, states that arrest of the heart is always preceded by arrest of the circulation. With respect to the action of chemical stimuli Schenk found with 72 hour hearts that dilute solutions of both acids and alkalis increased the frequency of their rhythm, while distilled water, a 2% solution of boric acid, and a 1% solution of sodium chloride as well as the vapour of ammonia to stop the heart. He concludes that "alle chemischen Agenten die Contraction des Herzens unterbrechen." Chemical excitation has also been taken up by Preyer, who states that nitrate of potassium is a violent poison to the embryonic heart, while the nitrates of sodium and of ammonium are innocuous. He also states that atropine, hydrate of chloral, quinine and nicotine all paralyse the embryonic heart, while alcohol and ether in small doses augment the frequency of the embryonic heart, but finally stop it in diastole.

As will be subsequently shewn in the experimental portion of this paper, the doses of Schenk and of Preyer were too large to produce any other than a depressing effect.

The latter observer found that moderately strong induced currents increase the frequency of the embryonic heart, while stronger currents diminish the duration of the diastoles and finally produce a systolic stoppage.

A constant current he states to have no effect upon the hearts of chick embryos, though he has noted an increase of frequency when it is applied to those of guinea-pig embryos.

Fano⁽²³⁾ believes that the automatism and excitability of the embryonic heart are independent of each other and that the excitability to stimuli increases with the age of the heart, while it *pari passu* loses its power of resisting lesions. His method, which will be discussed more fully later on, consisted in excising the embryonic heart from its blastoderm and keeping it on a warm stage. By the aid of photographic apparatus in collaboration with F. Badano⁽²⁴⁾ he has measured the time taken by the peristaltic wave to pass over the embryonic heart, and finds that it varies with the condition of the heart. In freshly extirpated hearts its highest limit was .080 of a second, which if the hearts have been longer extirpated may fall to .250 of a second. There is a difference in the length of the concave and convex borders of the heart and the

wave of contraction which passes over the embryonic heart at a rate varying from 11.5 to 3.6 mm. per second, takes longer to travel along the convex than along the concave side from which Fano concludes "que la vitesse de transmission de l'onde pulsatoire est égale à elle-même dans toute la masse du cœur." Preyer stated that he had observed simultaneous contraction of the different portions of the embryonic heart. As the result of a large number of experiments on this point Fano writes, "or nous n'avons jamais observé, ni directement, ni dans nos traces, le fait relevé par Preyer de la contraction simultanée de points différents du cœur." From these observations he concludes that the propagation of the contractile wave of the embryonic heart is direct from cell to cell of its substance.

These observers also divided the heart into several portions and noted that after dividing the auricle from the ventricle, the former continues to contract, while the ventricle stops, but resumes beating after a varying period of quiescence, in one case 15 minutes. The auricular rhythm after this operation is more frequent than the ventricular rhythm. Experimenting with gases they found that oxygen accelerated the rhythm of the heart and that hydrogen and carbonic oxide diminished it, while carbonic dioxide arrested the heart almost instantly.

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(20) Schenk. Sitzungsberichte of the Vienna Academy. Bd. lvi. (1867), p. 111.

(21) Cleland. Journal of Anatomy and Physiology. Vol. xi. (1877), p. 754.

(22) Daresté. Association Française pour l'Avancement des Sciences, 7^e Session (1878), p. 741. "Comptes Rendus," T. lxxxvi. (1878), p. 725.

(23) Fano. Lo Sperimentale. 1885.

(24) Fano and Badano. Archives Italiennes de Biologie, T. xiii. (1890), p. 387; also in Archivio per le Scienze Mediche. Vol. xiv. p. 113.

On the methods adopted in the examination of the Embryonic Heart.

Of the early observers Harvey seems to have examined the embryonic heart *in situ*. Cleland floated the blastoderm under examination on to a watch-glass and by pouring off the water left the embryo adherent to the watch-glass. Fano opened his eggs in 75% saline solution and detached the embryo by a circular cut around the area pellucida. The embryo was then transferred to a warm slide, where its heart was dissected out and affixed by two needles to the slide. Means were taken to prevent loss of moisture. This method has the advantage that by it a photographic record of the wave of contraction passing over the heart can be obtained. It is however

open to the objection that there is, owing to the abnormal conditions under which the heart is observed, a gradual loss of energy of the heart, which renders it an unsuitable method for studying the action of drugs and varying conditions when they are acting over comparatively long periods of time. This method though eminently suited for Fano's purpose is unsuited for mine.

Preyer and those who worked under him examined their embryos by two methods:

(a) They decalcified the egg-shells by chemical agents and examined the embryos *in situ* by the aid of strongly reflected light.

(b) They opened the tops of the eggs and kept them warm by plunging them in sand, which in turn was heated by a water-bath.

When they were not making observations a metal lid excluded the embryo from the influences of the surrounding atmosphere, to which it was exposed during the time of the observations. To avoid the inaccuracies his pupil Wernicke made observations on the frequency of the heart rhythm during the 30 seconds which followed the opening of the egg.

The aim of the method I have adopted has been to disturb to the least possible degree the normal condition of the embryo. To attain this object I have devised the "observing incubator" figured at the end of this paper. It consists of a water-bath made of copper, surrounding on five sides an air chamber. The temperature of the air chamber can by the means of the water-bath be kept constant, or varied at pleasure. The remaining upper side of the air chamber is covered by glass.

Suspended within the air chamber by means of copper strips and binding-screws is the egg under observation. The copper strips are supported by a wooden frame which rests upon the top of the incubator and which bears the glass top as shewn in the diagram. By this means but little heat is conducted from the copper sides to the egg. Two thermometers resting upon the wooden frame record the temperature of the air of the chamber.

A window 3 cm. square is cut through the shell and shell membrane of the egg under observation, the albumen and the blastoderm being undisturbed. The egg and contained embryo are then placed in the "observing incubator" the atmosphere of which is kept moist by the evaporation of water from a small bowl placed within it. Preyer has pointed out that the evaporation of water from the surface

over the embryonic heart depresses its rhythm, and the method adopted prevents the intervention of this source of error.

If the temperature be kept constant the rhythm of each individual embryo will remain constant for many hours if kept in the apparatus described above. The shock of opening the egg due to the exposure of the blastoderm to the cold air of the laboratory will often cause depression of the rhythm, which may persist for two or even three minutes, after which time if placed in the "observing incubator" the embryonic heart will assume a regular rhythm.

Observations made during the thirty seconds which follow the opening of the egg are therefore liable to error. It is also advisable to keep the egg for 10 or 12 minutes in the incubator before commencing to record observations. In order to administer chemical stimuli it is necessary to find some solvent which itself has no influence on the embryonic heart. This is furnished by 65% sodium chloride solution, which if administered at the temperature of the embryo does not influence its cardiac rhythm. For this reason all drugs have been dissolved in this solution and given at the same temperature as the embryo. Distilled water depresses the cardiac rhythm. It is therefore unadvisable to administer drugs simply dissolved in distilled water as some observers have done. All substances have, except where otherwise stated, been administered by direct application to the heart by the means of a hypodermic syringe.

On the Normal Rhythm and its Modifications.

The normal contraction wave passes from the entrance of the veins into the auricle, and thence to the ventricle. If a small piece of fine silk fibre be placed at the auriculo-ventricular junction, partial blocking of the auriculo-ventricular sequence can be obtained, so that for every two or three auricular beats, only one beat of the ventricles results. If the rhythm be stopped by allowing the air of the observing incubator to slowly cool to 10° C. or lower, it often results that the auricular portion of the heart continues to beat after the ventricles. The auricular myoplasm is therefore endowed with a higher power of spontaneity than the ventricular myoplasm. If now the auricle be gently stimulated by a needle point, the increased auricular beat spreads to the ventricle, which contracts in the normal sequence. The same result ensues from the application of a weak interrupted electrical current. If both auricle and ventricle have passed into complete standstill,

stimulation of the auricles will, if the standstill has not been too long, lead to contraction both of the auricle and the ventricle. A stimulation either electrical or mechanical of the ventricular end leads to a wave of contraction which commences from the auricular end and follows the normal sequence from auricle to ventricle. This shews direct conduction in the substance of the ventricular and auricular myoplasm, since the wave of contraction does not originate from the point of stimulation.

I may here mention that the contraction of the embryonic heart even when under profound modification, due to the actions of poisons, always occurs as a peristaltic wave. Like Fano¹ I have never observed the simultaneous contraction of the different parts of the embryonic heart said to exist by Preyer². Duval³ has shewn that in embryos from 60 to 80 hours the auriculo-ventricular valve is absent and represented by an infundibuliform slit. I have examined transverse sections of 72 hour embryos and find that the elongated cells of the cardiac myoplasm are arranged in a longitudinal manner at the point of junction of the auricles and ventricles. There is at this period no defined auriculo-ventricular ring of fibres. Yet if the normal rhythm be impaired by the action of a depressant poison like morphine or a salt of potassium there is a marked pause between the contraction of the auricle and that of the ventricle. Also in cases when warming the heart will cause it to recommence beating, the auricle often begins before the ventricle, and finally its beats spread to the ventricle.

Foster⁴, writing of the sino-auricular and auriculo-ventricular pause in the rhythm of the frog's heart, has stated "we may thus consider the breaking of the primitive unbroken peristaltic wave of contraction from sinus to bulbus to be due to the introduction of tissue of lower conducting power at the junctions of the several parts." It seems however that in the embryonic heart the "lower" activity of ventricular myoplasm is the cause of the auriculo-ventricular pause, the ventricle apparently taking a longer time to respond to the stimulus of the contraction wave reaching it from the auricles, than do the individual cells of the auricular myoplasm itself. If it is safe to apply conclusions drawn from the myoplasm of the embryonic heart to the adult heart it would seem that one factor in determining the auriculo-ventricular pause is the "lower" activity of the ventricular fibre generally. Whether there is any special

¹ Fano. *Op. cit.*

² Preyer. *Loc. cit.*

³ Duval. *Les travaux de lab. de Physiol. de Paris.* T. 1. (1885).

⁴ Foster. *Text Book of Physiology*, (5th ed.), p. 289.

introduction of fibres of lower conducting power at the auriculo-ventricular junction, or whether the pause in the rhythm between the auricles and ventricles is wholly due to the lower activity of the ventricular fibre can only be determined by direct experiment on the adult heart, where it might be observed if the auriculo-ventricular junction were less responsive to stimuli than the remainder of the ventricular fibre.

A fundamental question in determining the variations produced by external agencies on the embryonic heart is whether there is a normal rhythm? By the term normal rhythm I mean a rhythm of common frequency for hearts of the same age when under the same external conditions. Reference back to my historical summary will shew that different observers have given different "normal" frequencies for the embryonic heart, the widest divergence being Remak with 40 systoles per minute and Von Baer with 150 per minute. The absence of a truly normal rhythm is also well shewn by Wernicke's¹ table, who in three-day embryos obtained variations of frequency between 90 and 146 beats per minute in the 10 eggs of that age which he examined. I have in my section on the methods adopted pointed out my reasons for believing that Wernicke's method was liable to error. I have examined some 250 embryonic hearts at ages varying from 60 to 100 hours, and over 150 at 72 hours' incubation (at the temperature of 38° C.), under the same conditions of temperature and moisture, with the precautions cited in my section on method. From these observations I conclude that there is no constant frequency in embryonic hearts of the same age and under the same conditions but each embryo has an individual rhythm of its own, which if the external circumstances are unvaried remains constant. It is therefore necessary to determine the constant rhythm of each embryo before studying the variations which result from stimuli.

The frequency which in each experiment that follows I have termed the normal rhythm is really the normal individual frequency under the conditions of temperature stated in the particular experiment. The remaining conditions, such as moisture, are constant in each and every experiment, where the normal rhythm was determined.

Action of Variations of Temperature.

The following experiment illustrates my results with varying temperatures.

¹ Wernicke. *Loc. cit.* p. 30.

In an embryo aged 72 hours, an experiment which lasted four hours was commenced at 34° C., at which temperature its cardiac rhythm was 85 beats per minute. The temperature of the air of the incubator was rapidly raised to 41° C., when the rhythm became 91 beats per minute. A further increase of the temperature of the air of the incubator to 50° C. raised the cardiac rhythm to 129 beats per minute, but owing apparently to a condition of tonic contraction having set in, the beats were reduced in force. The temperature was then rapidly lowered to 26° C., and the rhythm almost immediately fell to 115 beats per minute, while the condition of idiomuscular contraction disappeared. A further rapid fall of temperature to 18° C. reduced the rhythm to 34 beats per minute, while the systoles were apparently weakened, the whole heart being more expanded than in the normal condition. The temperature was then rapidly raised to 46° C., when the rhythm rose to 117 per minute. A second fall of temperature to 29° C. reduced the rhythm to 56 per minute.

In other experiments with embryos of ages varying from 50 to 120 hours similar results were obtained.

The preceding observations when considered with those quoted from Harvey, Eckhard, Schenk, Wernicke, Preyer, Cleland and Dreste, shew that heat acts directly upon the activity of the embryonic myoplasm, increasing its activity within certain limits, above and below which it becomes a depressant. This is but another instance of the many examples which prove that heat modifies the metabolism of all contractile tissues. Thus similar alterations of rhythmic action have been shewn by the following investigators in the types named, by Romanes¹ in medusæ, by Newport² in insects' hearts, by Carus³, Brandt⁴ and Plateau⁵ in crustacean hearts; by Yung⁶ in molluscan hearts; by Dolguel⁷ in the heart of the larva of *Corethra plumicornis*, by many observers⁸ on the batrachian heart, and recently

¹ Romanes. *Croonian Lecture Roy. Soc.* Dec. 1st, 1875, (Vol. 166).

² Newport. Article "Insecta" in *Todd's Cyclopaedia*. Vol. vii. (1839), p. 981.

³ Carus. *Von den äussern Lebensbedingungen der heiss- und kaltblütigen Thiere*. Leipzig, 1824, S. 84.

⁴ Brandt. *Physiol. Beobachtungen am Herzen des Flusskrebses*.

⁵ Plateau. *Archives de Biologie (Van Beneden's)*. T. 1. (1880), p. 633; *Bulletin de l'Acad. Roy. de Belgique*, 2^{me} série. T. 41, p. 107.

⁶ Yung. *Archives Expér. de Zool.* T. ix. (1881), pp. 429—444.

⁷ Dolguel. *Mém. Acad. de Pétersbourg*, vii^e série. No. 10 (1877), p. 16.

⁸ A historical account, including the early observations of Humboldt, Panum, Pickford, Cyon, Budge and Tigger, with references, together with his own work on the subject is given by Brnnton in *St Bartholomew's Hospital Reports*, Vol. vii. (1871),

by Newell-Martin¹ on mammals' hearts; as well as on the contractile power of the uterus, vas deferens, digestive tube, vagina and on cilia by Calliburcès². From these observations I conclude that the rhythmic activity of contractile tissues varies with their temperature.

Small variations of temperature³ if taking place over a comparatively long period of time, as for instance a change of one or two degrees in two or three hours, do not influence the cardiac rhythm of a carefully kept embryo.

Newell-Martin (*loc. cit.*) believes that variations in the body temperature during fever may account for the variations in the pulse-rate and cardiac rhythm that are observed under such conditions. Is it not however more probable that such temperature variations being comparatively slowly produced during the febrile condition do not materially influence the cardiac rhythm, but that any changes in the rhythm that are observed under such conditions are due to the circulation of toxines in the vascular system?

As Plateau⁴ found on the heart of the lobster so I find that the heating of the embryonic heart above certain limits enfeebles the rhythm by diminishing the length of the systoles, while the diastoles become imperfect. The extreme feebleness of the rhythm is shewn by the anæmic condition of the whole blastoderm. Periods of rapid beating alternate with periods of rest. In some cases the stoppages were in systole, but the final fatal stoppage of the overheated embryonic heart was in diastole. According to Preyer⁵ a heat tetanus does not exist in the embryonic heart. The absence of a graphic method renders it extremely difficult if not impossible to decide whether there is or is not a tetanic fusion of the heart-beats of the embryo under the influence of high temperatures, and it is possible to explain the shortened systoles as an "idiomuscular contraction," and the whole condition of the heart as comparable to that described by Kronecker⁶ as "Herz Delirium."

p. 216; for more recent work see René, *Archives de Physiologie*, Vol. xxii. (1890), p. 596, and G. N. Stewart, *This Journal*, Vol. xiii. (1892), p. 59.

¹ Newell-Martin. *Phil. Trans. Roy. Soc.* 1883, (Croonian Lecture), Vol. 167, p. 689.

² Calliburcès. *Comptes Rendus.* T. xlvi. (1858), p. 638.

³ Even up to 5° C.

⁴ Plateau. *Association Française des Sciences.* 1878, p. 734.

⁵ Preyer. *Op. cit.* p. 31.

⁶ Kronecker. *Beit. zu Anat. und Physiol.* 1874.

The final relaxation which succeeds the contracted phase seems to point to the latter explanation. This final diastolic stoppage as the result of overheating agrees with the observations of Aristow¹ and of Stewart², who describe a final diastolic heat stoppage in the frog's heart.

On the Action of Drugs of the Caffeine Group.

We shall consider under this head—Caffeine, which is trimethyl-xanthine, theobromine, which is dimethyl-xanthine, and xanthine itself³ in their action upon the cardiac myoplasm of the embryo and allied contractile tissues.

A. Caffeine.

Additional interest has been attached to the action of this drug, since as before stated, Loew⁴ has found it to combine directly with certain portions of protoplasm.

Albers⁵ believed that caffeine caused tetanus even of the heart fibre. Stuhlmann⁶ and Falck observed acceleration and irregularity of the heart's action under the influence of caffeine. Voit⁷ noted an increase of action followed by a decrease after its administration. Detel⁸, Penilleau⁹, and Prompt¹⁰ found that caffeine accelerated the pulse. Leven¹¹, who made extensive experimental researches, concluded that the primary acceleration only gave way to depression when fatigue of the cardiac muscle set in.

Méplain¹² however concluded that the primary acceleration was absent; and there was only a diminution of rhythmic power. Batek¹³ has recently confirmed Leven's views. Bennett¹⁴ made comparative

¹ Aristow. *Archiv für Anat. und Physiol.* (Phys. Abth.) 1879, S. 198.

² G. N. Stewart. *This Journal.* Vol. xiii. (1892), p. 122.

³ For the chemical relations of these bodies see E. Fiseher, *Liebig's Annalen.* Bd. ccxv. (1882), S. 253. *Ber. d. deut. chem. Gesell.* Jahrg. 14, S. 637; Jahrg. 15, S. 290, u. 453.

⁴ Loew. *Notice sur le Deuxième Congrès de Physiologie.* Liège, 1892, p. 32.

⁵ Albers. *Deutsche Klinik red. von Goeschen.* 1852, No. 51.

⁶ Stuhlmann and Falek. *Virchow's Archiv.* Bd. xi. (1857), SS. 324, 481.

⁷ Voit. *Untersuchungen über den Einfluss des Kaffees und der Muskelfixegungen auf den Stoffwechsel.* Münich, 1860.

⁸ Detel. *Thèse Inaug.* Paris, 1861.

⁹ Penilleau. *Thèse de Paris.* 1864.

¹⁰ Prompt. *Archives générales de médecine.* 1867, p. 358.

¹¹ Leven. *Archives de Physiologie.* 1868, p. 179.

¹² Méplain. *Thèse de Paris.* 1868.

¹³ Batek. "O Coffeinu" (Russian), abstract in French in the *Archives Slaves de Biologie.* T. i. (1886), p. 483.

¹⁴ Bennett. *Edin. Med. Journ.* Vol. xix. (1873), p. 323.

experiments with caffeine, theine, and theobromine on frogs and rabbits, and found first an increase and finally a decrease of action with each of these bodies.

The present condition of our knowledge is illustrated by Bradford¹ in his article in "Ringer's Therapeutics," in which he describes "a diminution in the force of the cardiac beats, this is followed by a period during which the heart-beats are slowed but markedly strengthened, to be followed in turn by a period of acceleration, during which the blood-pressure regains or even exceeds its former level. The action of the drug on the heart is probably a mixed one, not only does it cause a diminution of the force of the beats, but it probably at the same time causes a contraction, tonic in character, of the ventricular muscle. As a result of this the capacity of the heart is diminished, and hence a smaller quantity of blood will leave the organ at each systole." This agrees with Roy's² classing of caffeine amongst those drugs that produce "idiomuscular contraction."

Bradford and Phillips³ also write "we have, however, been unable to satisfy ourselves that the drug produces acceleration of the heart after section of the vagi." They also consider the diminution of the cardiac beats is due to inhibition phenomena on the medulla, since they are absent after section of the vagi.

The following experiments illustrate the action of caffeine on the embryonic heart.

(1) Nov. 1st, 1892. Embryo aged 70 hours, with normal rhythm of 88 per minute. Temperature throughout the experiment 35° C.

At 2.17.0 1 c.c. of normal saline containing '00015 gr. of caffeine was administered.

2.19.0 The rhythm was 100 per minute and normal.

The rhythm was retained at 100 per minute during the next four minutes and exhibited no peculiarity except that the heart was in slight tonic contraction.

2.23.15 The heart was in the same condition, but the frequency had fallen to 96 per minute.

2.24.15 Rhythm same as previous observation. A second dose of '00015 gr. administered.

2.26.25 Rhythm was 98 per minute, no visible change in heart.

2.27.25 Rhythm had risen to 102 per minute.

¹ Bradford. *Ringer's Therapeutics.* p. 527.

² Roy. *This Journal.* Vol. I. p. 477.

³ Bradford and Phillips. *This Journal.* Vol. VIII. (1887), p. 122.

2.28.0 Rhythm had fallen to 100 per minute.
 2.30.0 Rhythm had fallen to 90 per minute.
 2.31.0 Rhythm had fallen to 88 per minute. A third dose of .00015 gr. given.
 2.33.0 Rhythm rose to 92 per minute. The whole heart was more contracted.
 2.34.0 Rhythm 96 per minute.
 2.40.30 Rhythm 80 per minute. Heart still contracted, but not to such an extent as to interfere with its action.
 2.42.0 Rhythm 78 per minute, .0003 gr. given.
 2.44.0 Rhythm 92 per minute. Heart more contracted.
 2.46.30 Rhythm fell to 42 per minute. Heart in tonic contraction.
 2.47.10 A dose of .0006 gr. given.
 2.48.25 Rhythm was 52 per minute.
 2.49.25 Rhythm was 76 per minute.
 2.50.25 Rhythm was 76 per minute.
 2.51.30 A dose of .00075 was given, which was fatal in strong systole.

(2) Nov. 9th, 1892. Embryo aged 75 hours. Temperature during the experiment 37° C. Normal rhythm was 116 per minute.

At 1.51.0 1 c.c. of normal saline containing .0025 *caffeine* was given.
 1.52.0 The rhythm was 100 and very strong.
 1.52.45 The heart stopped in strong systole.
 1.53.0 The heart recommenced beating.
 1.53.30 During these 30 seconds there were 12 beats, each having a strong systole.
 1.54.5 During these 30 seconds there were 6 beats, followed by stoppage in strong systole.
 2.2.35 The auricular half of the heart gave two strong beats. The whole heart then passed into strong contraction and was irresponsible to stimuli.

These experiments shew a general correspondence of results between the embryonic heart and those recorded by Bradford and Phillips on the adult heart. There is induced in the embryonic heart the condition of tonic contraction, described by these observers and by Roy. The primary acceleration observed with the smaller doses corresponds to the primary acceleration cited by several observers in the adult heart. It is therefore probable that the chief phenomena described in the foregoing abstract as characterizing the action of caffeine on the adult heart are the result of the direct action of the caffeine on the cardiac fibre; and that the nervous elements play only a secondary part.

The fact that Bradford and Phillips were unable to obtain a definite acceleration after section of the vagi in their experiments seems to point to the action of nervous influences via these nerves, especially as caffeine is known to be a stimulant to nervous tissues. If therefore there is any nervous influence its action is greatly aided by the primary acceleration which the caffeine probably produces by its direct action on the cardiac fibre. To summarize—All the phenomena having their analogue in the action of caffeine on the embryonic heart, it is unnecessary to introduce a nervous hypothesis to explain the action of caffeine on the adult heart.

Experiment 2 illustrates a fact often observed that a much larger dose may be administered in portions than can be given at one dose. Thus in Exp. 1 '00135 gr. was given in small doses, with a resulting depression of only 10 beats per minute, while in Exp. 2 a single dose '0025 gr. was fatal after one minute forty-five seconds action. A condition of toleration is set up by small doses of the drug. Also as the result of a larger dose the tonic or idiomuscular contraction is very marked, with the result that the blood output is reduced. This is shewn by the anæmic condition of the whole blastoderm. In discussing the action of drugs on the heart, physiologists lay great stress on the reaction of the blood pressure upon the endocardiac pressure and thus upon the activity of the cardiac fibre. This action is well shewn by the hypertrophy of the cardiac fibre which results from increased arterial tension¹: but it is also possible that too much importance has been attributed to this factor, since in the blastoderm we can have no increased arterial tension, and yet the condition of the heart undergoes the same changes as when an increased arterial pressure is present. This remark also applies to drugs which stop the heart in dilatation (as chloroform) where the dilatation is supposed to directly result from increased blood pressure. There is a striking difference between the action of caffeine on the contractile tissue of the medusæ and the cardiac fibre and myoplasm, as is shewn by the researches of Romanes², for although there is a primary acceleration of the rhythm, yet at the same time the beats diminish in potency, then the contractions gradually fade away and the animals lose their tonus and become relaxed to the utmost extent. This relaxation will even be produced if a medusa that has been

¹ Vide Ziegler's *Pathology* (translated by Macalister). Part II. p. 49 (1887).

² Romanes. *Phil. Trans.* Vol. 167, part II. p. 736.

anæsthesized (and consequently had the functional activity of its nervous mechanism temporarily abolished) be placed in a solution of caffeine. Since the relaxed phase of the medusa's rhythm corresponds to the diastolic stage of cardiac activity, there is therefore a fundamental difference between the medusa's rhythmic contractile tissue, which on account of its primitive organisation may to some extent represent persistent ancestral contractile tissue, and the embryonic myoplasm. We might anticipate a similarity between embryonic contractile tissue and this primitive contractile tissue, and we have apparently a physiological exception to the famous law of Kowalewsky¹ that—every individual in its embryonic development tends to repeat its ancestral development. That this divergence of action is not due to the nervous rete present in the subumbrellal tissue of the medusæ is shewn by its production in the anæsthesized animal. Possibly the difference of action is the result of a fundamental difference of metabolism, since the environment of the two classes of contractile tissue under consideration is essentially different. From this fact it also may be pointed out that it is undesirable to reason from the action of drugs and other stimuli on protoplasm that has retained a primitive ancestral condition, that a similar action will result on more differentiated tissues, and, as corollary to this, it is not improbable that persistent naked protoplasm and comparatively undifferentiated contractile tissue (shewing these physiological differences) have a different chemical composition, which is correlated with the varying physiological action. There are however similarities between simple protoplasm and the higher contractile substances; thus quinine, according to the original observations of Binz², entirely arrests the movements of leucocytes, while Geltowski³ has shewn that this action will take place even when the dilution of the quinine is 1 in 1500.

Preyer⁴ states that quinine is the most toxic of all the agents he has applied to the embryonic heart, while the hypodermic injection of 3 to 12 centigrammes of quinine in a frog will rapidly paralyse the muscular substance of its heart (Jolyet⁵).

¹ Kowalewsky. See researches in *Acad. des Sciences de S. Petersbourg*, commencing VII^e série, tome x. 1866.

² Binz. *Virchow's Archiv.* Jahrg. 1867 und 1868. *Berlin Klin. Woch.* 1871.

³ Geltowski. *Practitioner.* Vol. VIII. p. 325—330.

⁴ Preyer. *Op. cit.*

⁵ Jolyet. *Comptes Rendus.* T. LXIV. (1867), p. 719 et 421.

B. *Xanthine.*

My results with xanthine are illustrated by the following experiment.

Nov. 18th, 1892. Embryo aged 96 hours. Temperature throughout the experiment 33° C. Normal rhythm 102 per minute.

At 12.11.0 1 c.c. of normal saline containing .0004 *gram of xanthine* was administered.

12.13.0 Rhythm was 102, and apparently normal in every respect. At 12.14.0 the heart was in the same condition.

12.15.0 No change in condition of the heart. A second c.c. of *xanthine* solution containing .0004 *gr.* was given.

12.21.0 The rhythm at this time was normal, having remained unchanged from that of the preceding observation.

12.25.0 Rhythm still normal. 3 c.c. of solution given containing .0012 *gr. of xanthine.*

12.26.0 Frequency 80 per minute. Rhythm otherwise normal.

12.27.0 Frequency 80 per minute, no change in rhythm.

12.32.0 Frequency 92 per minute, no change in rhythm.

12.34.0 Frequency 96 per minute, no change in rhythm.

12.36.0 Frequency 96 per minute, no change in rhythm.

12.37.0 No change in frequency or rhythm. 3 c.c. of solution containing .0012 *gr. of xanthine was given.*

12.37.0 No change in rhythm.

12.38.0 No change in rhythm.

12.40.0 No change of rhythm. 1 c.c. of solution containing .0012 *gr. of xanthine was given.*

12.41.0 Frequency 98. No other change observed.

12.42.0 Frequency 98.

12.44.0 Frequency 102 per minute. Beats were strong.

12.45.0 Frequency 116 per minute. Beats were strong.

12.46.0 Frequency 120 per minute. Beats apparently not so strong.

12.47.0 Frequency 114 per minute.

12.48.0 Frequency 115 per minute.

12.49.0 Frequency 108 per minute.

12.50.0 Frequency 104 per minute.

12.51.0 Frequency 104 per minute. 1 c.c. containing .0012 *gr. of xanthine given.*

12.52.0 Frequency 126 per minute. The strength of beats apparently weakened.

12.53.0 Frequency 122. Force as above.

12.54.0 Frequency 126. Force as above.

12.55.0 Frequency 122. Force as above.

12.56.0 1 c.c. of solution containing '0012 gr. of xanthine given.
 1.1.0 Frequency 120. No change.
 1.2.0 Frequency 120. No change.
 1.3.0 Frequency 120. No change.
 1.4.0 Frequency 120. Commencing weakness of the systoles.
 1.22.0 Frequency 102. Beats weaker than in normal rhythm.
 1.23.0 1 c.c. of solution containing '0012 gr. of xanthine added.
 1.24.0 Frequency 86. Beats only slightly weaker than the normal.
 1.30.0 Heart in the same condition.

This experiment shews that xanthine caused a primary depression of the embryonic heart, followed by an acceleration and an increase in the strength of the beats. Very large doses slightly weaken the rhythmic power of the heart, but I have been unable to induce any tonic contraction.

C. Theobromine.

The following is an example of the action of theobromine.

Nov. 19th, 1892. Embryo aged 70 hours. Temperature throughout the experiment 31° C. Normal rhythm 112 per minute.

At 12.52.45 1 c.c. of normal saline solution containing '0005 gr. of theobromine was administered.

12.54.45 The frequency and characteristics of the rhythm remained normal.

12.57.0 The frequency and characteristics of the rhythm still normal. '00075 gr. of theobromine in 1 c.c. of normal saline was given.

12.58.0 Frequency rose to 120 per minute. The characteristics of the rhythm remained normal.

12.58.0 Same as above record.

1.2.10 Frequency 140 per minute. Beats apparently very strong.

1.3.10 Frequency 150 per minute. Beats apparently very strong.

1.5.0 Rhythm unchanged. '0005 gr. of theobromine given.

1.10.0 Rhythm unchanged. '00075 gr. of theobromine given.

1.12.0 Rhythm unchanged.

1.13.0 Frequency 136 per minute. Diastoles somewhat imperfect.

1.14.45 Frequency 140 per minute. Diastoles slightly imperfect.

1.16.30 Rhythm as in preceding observation, '0015 gr. of theobromine given.

1.20.0 Frequency 112 per minute. The rhythm did not show any peculiarities from the normal.

1.22.0 Frequency 126 per minute. '0005 gr. of theobromine given.

1.24.0 Frequency 124 per minute. The characteristics of the rhythm remained normal.

1.26.0 A further dose of .0015 gr. of theobromine was given.

1.30.0 Frequency 130, and rhythm otherwise normal.

1.32.0 Observation same as above. 2 c.c. of saturated solution of theobromine was given.

1.35.0 Frequency 124, and rhythm otherwise normal.

1.38.0 Frequency 140, and rhythm otherwise normal.

1.42.0 Observation as preceding.

1.44.0 Observation as preceding.

1.45.0 2 c.c. of saturated solution of theobromine given.

1.48.0 Frequency 140, and rhythm otherwise apparently unchanged.

1.50.0 Frequency and rhythm unchanged.

1.51.0 2 c.c. of saturated solution of theobromine given.

1.55.0 Frequency 120, and rhythm otherwise normal.

The experiment recorded above shews that theobromine in doses of .00075 gram will greatly increase the frequency of the cardiac rhythm of the embryo. Larger doses apparently have little or no depressant effect, since the frequency was maintained at 120 per minute, or 8 beats per minute above the normal frequency, after more than .006 gram had been given. There was even after these doses no alteration to be detected in the character or force of the cardiac rhythm. During the primary phase of acceleration the systoles of the heart were somewhat stronger than the normal. There was however no condition of tonic contraction during any phase of its action.

D. General considerations respecting the Caffeine group.

The close chemical relationship of the three substances considered in the foregoing section, and their difference consisting of the introduction of methyl groups, as well as the occurrence of xanthine in the organism, makes them peculiarly adapted to the study of the relationship between the physiological action and the chemical composition of stimulus employed. In this connection I may remark that xanthine contains a (CONH) atomic group, and that there are some reasons for believing that a (CONH) group may exist in the proteid molecule¹. Further, the work cited from Loew and Bokorny tends to shew that a definite combination between naked protoplasm and caffeine may exist, so that further investigation may be carried on, on both chemical and physiological lines. I have in this paper restricted myself to the latter method.

¹ See author's paper. *This Journal*, Vol. xiv. (1893).

It will be seen that xanthine has no constricting action on the tone of the heart, it even tends to produce an atonic condition. Theobromine causes a slight increase of the cardiac tone, but even after the administration of a saturated solution the systoles were apparently normal and there was no visible idiomuscular contraction.

Caffeine is the drug which on the embryonic heart produces pronounced idiomuscular contraction. We may tabulate these results thus.

No methyl groups in the molecule.	Tendency to the production of an atonic condition.
Two methyl groups in the molecule.	Slight improvement of systoles but no pronounced tone.
Three methyl groups in the molecule.	Pronounced tonic contraction.

There being evidence that caffeine combines with contractile tissues, it is not improbable that the condition of tonic contraction produced by the application of caffeine is owing to the introduction of methyl groups into the proteid molecule.

It has been shewn by several¹ observers that the introduction of methyl or ethyl groups into certain alkaloids modifies their physiological action—often changing a convulsive action into a paralysing action. It will therefore be a question for future decision whether there is any relationship between tonic action and paralysis of motor nerves.

Bennett², who worked with theobromine and caffeine, did not distinguish any fundamental difference in their action. Fihelne³, who has recently tried their comparative action on frogs' hearts, has observed that marked differences exist between the action of caffeine and theobromine on different species of frogs⁴. He has also however observed a diastolic standstill to be a characteristic of their action.

For this reason I have carried out my experiments with one species of fowl's egg viz. Cochins, and have as stated obtained results in the case of caffeine closely corresponding to the action known to exist on mammals' hearts.

¹ Buchheim and Loos. *Eckhard's Beitrage*. Bd. v. also Brunton and Cash's recent paper. *Trans. Roy. Soc.* 1892.

² *Op. cit.*

³ Fihelne. *Archiv für Anat. u. Physiol.* (Du Bois Reymond's *Archiv*) 1886, S. 72.

⁴ See also Schmiedeberg's original paper, "On the difference of the action of caffeine on varying species of frogs." *Archiv für exper. Path. u. Pharm.* Bd. ii. S. 62.

On the Digitalin Group.

The action of digitalin, antiarin, helleborin, and strophanthin, are peculiarly interesting, since there is considerable evidence as to their direct action on adult cardiac fibres. The observations previously made have been complicated by the action on the muscular coat of the arteries which is obviously eliminated in these experiments. We have therefore the means of testing how far the myoplasm of the embryonic heart corresponds in its physiological reactions to the fully developed cardiac fibre. In order to lay the evidence before my readers I will briefly consider the chief steps by which our knowledge of the action of these drugs on the heart has been advanced.

Sir B. Brodie⁽¹⁾ found that upas antiar paste stopped the hearts of dogs and rabbits in distension. From a clinical point of view a large number of physicians, amongst whom were Mosman⁽²⁾, Kinglake⁽³⁾, and Winograd orff⁽⁴⁾, stated that digitalin slowed the heart.

Stannius⁽⁵⁾ experimented with digitalin on cats, rabbits, and other animals, and obtained with a large dose diastolic stoppage, the heart being irresponsible to stimuli. He believed that digitalin acted directly on the musculo-motor elements of the heart, but stated that it was impossible to determine whether the contractility of the muscle was or was not dependent on the nerves.

His chief opponent was Traube⁽⁶⁾, who attributed the slowing of the heart to stimulation of the inhibitory nerves or by action on a cardio-inhibitory centre. The increase of blood pressure he thought to be owing to a primary stimulation of the cardio-motor centre. A large number of observers, including Blake⁽⁷⁾, Legroux⁽⁸⁾, Briesemann and Boldt⁽⁹⁾, Gouvat⁽¹⁰⁾, Ackermann⁽¹¹⁾ and Böhun⁽¹²⁾, have shewn that the increase of blood pressure is due to constriction of the arterioles. This increase of pressure must react on the heart, influencing its rhythm, thus obscuring the direct action of the drug on that organ. It would also modify the elasticity of the heart and this again its work.

Working under Kölliker's superintendence Pelikan⁽¹³⁾ found that upas antiar acts directly on the heart, exclusive of any extrinsic nervous mechanism. Dybkonsky and Pelikan⁽¹⁴⁾ shewed the similarity of action of antiarin and digitalin. They obtained a primary acceleration of the heart rhythm. In toxic doses they observed irregularities and two types of paralysis. (a) The ventricular contractions became peristaltic, so that while one portion of the ventricle was

dilated another portion would be constricted, and vice-versâ. After further action the irregularities became more marked, so that even the auricles sometimes did not contract simultaneously. Finally, when the ventricles had stopped, small palpitating points were observed upon them. (β) Slow diminution both in force and frequency of the beats. This sometimes happened before, and sometimes after, a peristaltic phase.

Braidwood⁽¹⁵⁾ stated that there was a difference in the action of digitalin on the hearts of cold and warm-blooded animals. In cold-blooded types the ventricle always stopped in strong contraction, while in the warm-blooded forms the ventricle and auricle is always stopped on expansion.

Alfermann⁽¹⁶⁾ found with frog's heart and small doses of antiarin there was sometimes a small acceleration, and afterwards the irregularities set in.

Hilton-Fagge and Stevenson⁽¹⁷⁾ and Nunnely working with antiarin and digitalin confirmed Dybkonsky and Pelikan's work, and described the peculiar "pouching" of frog's ventricle which characterizes toxic doses. This is probably explained by local excitation of the cardiac muscular fibres, since Schiff and Rossbach and Mc William have obtained a similar phenomenon by a local stimulus.

Milner-Fothergill⁽¹⁸⁾ tried the action of infusion of digitalin upon minnows. Their ventricles became so strongly contracted that there was no visible cavity. The auricle was distended, but, on pricking it and allowing the escape of blood, passed into strong contraction. J. Müller⁽¹⁹⁾ found that in rabbits during the first stage of asphyxia the local action of .00005 gram of antiarin stopped the auricles almost immediately, while the ventricles continued to pulsate for another minute.

Boinet and Hédon⁽²⁰⁾ also have recently described the action of antiarin on the mammalian heart, and have shewn that it corresponds in many particulars to those previously described in the amphibia.

Dyon⁽²¹⁾ has however recently concluded from work on the mammalian heart, that the arrest in antiarin poisoning is due to many causes, and is sometimes through the medullary centres and at others direct on the cardiac fibre.

Schmiedeberg⁽²²⁾ found that if a frog's ventricle poisoned by antiarin or digitalin be forcibly distended the heart beats are restored. He concluded that the stoppage was due to failure of the post-systolic relaxation, and hence is due to its action on the elastic properties of cardiac tissue.

Foster⁽²³⁾ found that this recovery was only effected if the distension was performed directly after the stoppage, and that in lethal doses the changes are more profound. Gaskell⁽²⁴⁾, experimenting with heart apices, has shown that digitalin and antiarin act like an alkali, and thus confirms Roy's⁽²⁵⁾ view that a direct "idiomuscular contraction" has been induced.

Donaldson and Stevens⁽²⁶⁾ found that with digitalin and a normal blood pressure the work done by the heart is decreased, but that after a rise of blood pressure digitalin increases the work done by the heart. Dybkonsky and Pelikan⁽¹⁴⁾ shewed that the hellebores might be included in the digitalin group. This was confirmed by Hilton-Fagge and Stevenson⁽¹⁷⁾. More recently Williams⁽²⁷⁾ has experimented on the frog's ventricle with helleborein, and has concluded that the rise of blood pressure which occurs after its administration is due to increased work of the heart. He also believed there was a change in the distensibility of the ventricle by which it underwent a greater diastolic expansion. The doses given are not stated, and in connection with this supposed increase of distensibility we may mention that Roy includes helleborein in those drugs which induce an "idiomuscular contraction," while Tschistowitsch⁽²⁸⁾, experimenting with aqueous extracts of the root of the green hellebore, observed a diminution in the number of heart-beats. The systoles first became more energetic, then peristaltic with imperfect diastoles, and finally the ventricles were arrested in extreme systole, while the auricles, though in extreme dilatation, continued to beat for some time.

We can now pass to the action of digitalin on the embryonic heart.

A. Nov. 12th, 1892. Embryo aged 70 hours. Temperature throughout the experiment 30° C. Normal frequency 132 per minute. The digitalin used was Schmiedeberg's preparation.

At 1.10.0 $\frac{1}{4}$ c.e. of normal saline containing .000022 gr. of digitalin was administered.

- 1.13.0 Frequency 130 per minute, no visible change in the rhythm.
- 1.14.0 Frequency 130 per minute, no visible change in the rhythm.
- 1.15.0 Frequency 132 per minute, no visible change in the rhythm.
- 1.17.0 $\frac{1}{4}$ c.e. of normal saline containing .000022 gr. of digitalin.
- 1.20.0 Frequency 132, no visible change in the rhythm.
- 1.21.0 Frequency 132, no visible change in the rhythm.
- 1.26.0 1 c.e. of normal saline solution containing .00005 gr. of digitalin.
- 1.27.0 Frequency and rhythm normal.
- 1.28.0 Frequency and rhythm normal.

1.30.0 1 c.c. of normal saline containing .0001 gr. of *digitalin*.
 1.31.0 Frequency 92 per minute. Systoles very strong and complete, diastoles imperfect.
 1.32.0 Frequency 104 per minute. Systoles very strong and complete, diastoles imperfect.
 1.33.0 Frequency 104 per minute. Systoles very strong and complete, diastoles imperfect.
 1.34.0 Frequency 104 per minute. Systoles very strong and complete, diastoles imperfect.
 1.37.0 1 c.c. of solution containing .0001 gr. of *digitalin*.
 1.38.0 Frequency 100 per minute. Rhythm very strong.
 1.39.0 Frequency 56 per minute. Rhythm very strong. There seems to be idiomuscular contraction setting in, as the diastoles are imperfect.
 1.42.0 Frequency 54 per minute. Same remark as above for rhythm.
 1.44.0 Frequency 40 per minute. Systoles very pronounced, ventricle in tonic contraction, auricle distended.
 1.45.0 Heart stops in tonic contraction and is consequently very pale.
 1.46.0 2 c.c. of normal saline containing .02 gram of potassium chloride was administered, but failed to restore the heart. The heart remained in tonic contraction.

Another type of result is illustrated by the following experiment.

B. Nov. 13th, 1892. Embryo aged 85 hours. Temperature of experiment 35°. Normal frequency 120 per minute. The same preparation of digitalin used.

At 12.31.0 1 c.c. of normal saline containing .00005 gr. of *digitalin* was administered.

12.33.0 Frequency 114 per minute. Rhythm very strong.
 12.34.0 Frequency 146 per minute. The beats were also very strong both as to systole and diastole.
 12.36.0 Frequency 140 per minute. The systoles very complete. There is slight tonic contraction, since the diastoles are imperfect and the heart is pale.
 12.37.0 Heart stopped and does not respond to mechanical stimulation.

The result recorded above in experiment B is not nearly so commonly obtained as the result of experiment A¹. As it is best obtainable with embryos over 80 hours, I at first thought there might be some nervous mechanism involved. But the fact of the loss of irritability of the whole heart greatly supports its being a direct action on the muscle. Experiment A is in accord with the view that digitalin decreases the

¹ 2 results out of 10 experiments, i.e. 20%.

frequency and increases the force of the cardiac rhythm. The tonic contraction recorded agrees with the idiomuscular contraction Roy and Gaskell have described.

Thus the embryonic heart under the influence of Schmiedeberg's digitalin behaves itself like the ventricular tissue of the adult frog's heart. It is interesting to compare these results with those of Romanes⁽²⁹⁾ on the medusa *Sarsa*, where digitalin first quickens the swimming motions and then progressively enfeebles them till they become mere twitches, and the animal dies in strong systole. There are considerable resemblances of the action of digitalin on the contractile substances of the hydromedusan sarsa and on the myoplasm of the embryonic heart. The primary acceleration described in my experiment B would then correspond to the acceleration of the systole described as a primary action of digitalin on sarsa. There is also a resemblance to the results of the action of digitalin on the crustacean and molluscan heart. Thus Plateau⁽³⁰⁾ found that the injection of 5 milligrammes of digitalin into a lobster caused irregularities of its cardiac rhythm, followed by stoppage and strong systole, while Yung⁽³¹⁾, using smaller doses, obtained an increase of seven beats per minute. With larger doses he obtained results identical with those of Plateau. Similar depressing results (without a primary acceleration) were obtained with the heart of *Mya arenaria*. Primary acceleration followed by depression resulted on the application of digitalin to the heart of the larva of *Corethra plumicornis* (Dolguel⁽³²⁾).

*Strophanthin*¹.

To Pelikan⁽³³⁾ we owe the observation that alcoholic extracts of strophanthus act on the hearts of batrachians in a manner similar to the drugs of the antiarin group. This observer noted the peristaltic nature of the ventricular contraction, the stoppage of the ventricle in extreme contraction, the great distension of the auricles, as results of this form of heart poisoning.

It was however by the researches of Fraser⁽³⁴⁾, and independently those of Polaillon and Carville⁽³⁵⁾, that an accurate knowledge of its action was obtained. Experiments were made on frogs, snails, birds, rabbits and dogs, and in all cases there was observed dilatation of the auricular and contraction of the ventricular portion of the heart. They concluded that the extract of strophanthus was a poison which acted on

¹ The strophanthin used was of Merck's manufacture.

the cardiac muscle of these animals, and that any nervous action if present was secondary. Vulpian⁽³⁶⁾ has corroborated these views, especially as to the heart of *Helix pomatia*, which has additional interest since Sheridan-Lea⁽³⁷⁾ has, after careful histological examination, concluded that ganglion cells are absent in the heart of this animal. Polaillon and Carville⁽³⁵⁾ stated that *Rhizostomæ* (*Scyphomedusa*) are unaffected by as much as ten milligrammes of their extract of strophanthus, but as only three experiments were made, and the results are contradictory to those cited with digitalin on medusæ, it would be advisable to repeat the experiments. The recent and brilliant researches of Fraser⁽³⁸⁾ will be quoted in discussing the results here recorded, which are illustrated by the following two experiments.

A. Oct. 18th, 1892. Embryo aged 70 hours and kept throughout the experiment at 32° C. The normal rhythm was 132 per minute.

At 12.12.45 1 c.c. of saline solution containing .00006 gram of strophantin was administered.

- 12.13.45 Rhythm in every respect normal.
- 12.14.45 Rhythm in every respect normal.
- 12.15.45 Rhythm in every respect normal.
- 12.16.45 Another 1 c.c. containing .00006 gram of strophantin administered.
- 12.19.0 Rhythm in every respect normal.
- 12.36.10 Frequency depressed to 54 per minute, but both systoles and diastoles complete.
- 12.40.45 Frequency 62 per minute, rhythm slightly irregular, but both systoles and diastoles complete.
- 12.41.45 Frequency 50 per minute, rhythm slightly irregular, with strong systoles and complete diastoles.
- 12.43.10 Auricle beating regularly, ventricle in tonic contraction, with a few beats.
- 12.45.40 The auricular systoles weak, and in a distended condition. The ventricle in a state of tonic contraction, giving a few beats. The auricular frequency was 42 per minute.
- 12.48.10 The auricle gave during the minute 20 beats, with strong systoles and perfect diastoles. The ventricle was in tonic contraction.
- 12.50.0 Auricle gave 24 beats, with weak systoles. Ventricle in tonic contraction.
- 12.53.0 Heart stopped auricle in diastole, ventricle in tonic contraction.
- 12.54.0 Mechanical stimulus to the auricle in 10 beats, which did not spread to the ventricle.

12.56.0 A mechanical stimulus to the ventricular end of the auricle caused a few reversed and feeble auricular beats which did not spread to the ventricle.

1.6.0 The auricle still responds to mechanical stimulation beating either from the auricular or from the ventricular end, according to the application of the stimulus.

B. *Oct. 19th, 1892.* Embryo aged 70 hours, temperature throughout the experiment 33° C. The normal rhythm was 120 per minute.

At 12.18.0 1 c.c. of normal saline containing .00003 gram of strophanthin was administered.

12.19.0 Frequency of rhythm 102 but systoles very strong. Heart paler than is normal. The diastoles imperfect.

12.21.0 Frequency 108 diastoles only slightly weak, systoles very powerful, heart becoming paler than is normal at each contraction.

12.22.0 Frequency 120, rhythm normal.

12.23.0 Frequency 120, rhythm normal.

12.24.30 1 c.c. containing .00006 gram of strophanthin administered.

12.25.0 Auricles dilated and gave 24 twitches in 30 seconds, ventricle strongly contracted giving a few weak twitches. Auricle responds to mechanical stimulus which does not visibly influence the ventricle.

12.32.0 Auricles and ventricles fail to respond to mechanical stimuli. Auricle dilated, ventricle contracted.

It will be seen from the above that strophanthin in small doses diminishes the frequency but increases the completeness of the ventricular contraction of the embryonic heart. In larger doses of .00006 gram it causes irregularities of rhythm, dilatation of the auricle and tonic contraction of the ventricle, and finally in the last stage of toxic action, the auricle responds to a mechanical stimulus the contraction wave starting from the point stimulated but not spreading to the ventricle.

Fraser⁽³⁹⁾ has recently made exhaustive experiments on the action of strophanthin on frogs' hearts, both isolated and *in situ*. His results with that animal are very similar to those recorded above with the embryonic heart. Thus .001 gram of strophanthin applied directly to a frog's heart after nine minutes' action reduced a rhythm 44 per minute to 36 per minute the ventricular systoles becoming longer and the diastoles incomplete. After 15 minutes' action the ventricle passed into tonic contraction, and the auricles were distended but had ample movements.

After 41 minutes' action the heart failed to respond to mechanical stimuli. The auricles were dark and distended while the ventricles were small, pale, and contracted. In conclusion he writes "The consideration of the structures affected and of the pharmacological change induced in them, has rendered it very apparent that ONE of the structures is the cardiac muscle itself. It is directly acted upon by strophanthus and the chief result of its action is an increase of its contractility rendering systole more prolonged and perfect."

Considering these experiments and conclusion in the light of the action of strophantin on the embryonic heart there can be but little doubt that the actions described above of strophantin are due to direct action on the cardiac myoplasm in the embryo, and cardiac fibre in the adult frog's heart.

The action of strophantin and digitalin shews that the myoplasm of the embryo-chick is very similar to the adult cardiac muscle in its reaction to certain chemical stimuli.

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- (1) B. Brodie. Phil. Trans. Roy. Soc. Vol. ci. (1811), p. 196.
- (2) Mosman. Essay on Scrofula etc., with observations on Digitalin.
- (3) Kinglake. Cases and observations on Med. Efficacy of Digitalis. London, 1801.
- (4) Winogradoff. Year Book of Syd. Soc. 1862, Archiv für path. Anat. Bd. xxii. (1861).
- (5) Stannius. Archiv für physiol. Heilkunde. Bd. x. 1851, S. 177, 209. (Vierordt's Archiv.)
- (6) Traubc. Ann. d. Char. Krankenhauses. Bd. xi. S. 785.
- (7) Blake. Edin. Med. Gazette. No. 139.
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- (10) Gouvat. Gaz. Méd. de Paris. 1871.
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- (12) Böhm. Pflüger's Archiv. Bd. v. (1872), S. 153. Dorpater med. Zeitschr. Bd. iv. (1873), S. 64.
- (13) Pelikan. Beit. zur gerichtlichen Medizin Toxicologie. Würzburg, 1858, S. 169. Comptes Rendus de la Soc. de Biologie. T. iv. (1857), p. 159.
- (14) Dybkonsky and Pelikan. Comptes Rendus de la Soc. de Biol. (1861) 3^e série, T. m., p. 93. Gaz. Hebdomadaire de Médecine etc. T. viii. (1861), p. 578. Comptes Rendus de l'Acad. des Sciences. T. lxxi. (1861), p. 384.

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(16) Alfermann. *Inaug. Dissert.* Marburg, 1865.

(17) Hilton-Fagge and Stevenson. *Proc. Roy. Soc.* Vol. **xviii.** (1870), p. 46.

(18) Milner-Fothergill. "On digitalis." *Hastings Prize Essay.* 1871.

(19) Müller. *Inaug. Dissert.* Berne, 1873.

(20) Boinet and Hédon. *Archives de Physiologie* (Brown-Séquard's). 5^e série, T. **iv.** (1892), p. 500.

(21) Dyon. *Archives de Physiol.* T. **iii.** (1891), p. 373.

(22) Schmiedeberg. *Ludwig's Festgabe.* 1874, S. 222.

(23) Foster. *Jour. Anat. and Physiol.* Vol. **x.** (1876), p. 586.

(24) Gaskell. *Jour. Physiol.* Vol. **iii.** p. 58.

(25) Roy. *Jour. Physiol.* Vol. **i.** p. 452—496.

(26) Donaldson and Stevens. *Jour. Phys.* Vol. **iv.** (1883—4), p. 169.

(27) Williams. *Archiv f. exper. Pathol. u. Pharm.* Bd. **xiii.** p. 1.

(28) Tschistowitsch. *Archives Slaves de Biologie.* T. **iv.** (1887), p. 354.

(29) Romanes. *Phil. Trans.* Vol. **clvii.** Part **II.**, p. 736. *Jelly-Fish, Starfish and Sea-Urchins.* Internat. Science Series. Vol. **l.** p. 222.

(30) Plateau. *Bulletin de l'Acad. Roy. de Belgique.* 2^e série, T. **xlvi.** (1878), p. 211.

(31) Yung. *Archives de Zoologie Expérimentale.* T. **vii.** 1878, p. 480. *Ibid.* T. **ix.** (1881), p. 437.

(32) Dolgiel. *Mém. Acad. de St Pétersbourg.* 7^e série, T. **xxiv.** (1887).

(33) Pelikan. *Comptes Rendus de l'Acad. des Sciences.* T. **lx.** (1865), p. 1209.

(34) Fraser. *Proc. Edin. Roy. Soc.* 1872.

(35) Polaillon and Carville. *Archives de Physiologie.* T. **iv.** (1872), p. 523. *Ibid.* p. 695.

(36) Vulpian. *Comptes Rendus.* T. **lxxxviii.** (1879), p. 1293.

(37) Sheridan-Lea. *Observations made for Foster and Dew-Smith's paper in Proc. Roy. Soc.* Vol. **xxiii.** p. 318.

(38) Fraser. *Trans. Roy. Soc. of Edinburgh.* Vol. **xxxvi.** Part **II.** (1890—1), p. 388.

On the action of Nicotine.

The action of nicotine is interesting since Langley¹, and Langley and Dickinson² have shewn that this drug exerts a selective action on

¹ Langley. *Proc. Camb. Phil. Soc.* Vol. **vii.** (1890), part **ii.**

² Langley and Dickinson. *Proc. Roy. Soc.* Vol. **vii.** (1890).

nerve cells. The action of nicotine on the heart has been studied for many years. Sir B. Brodie¹ found that tobacco enemata in the dog and cat rapidly stopped the heart in diastole.

Rosenthal² experimenting with nicotine obtained with small doses a primary slowing of the heart-beat, the length of the diastoles being greatly increased (sometimes lasting even one minute). Automatism is recovered while the inhibitory fibres of the vagus are still paralysed.

Jullien³ also observed the primary acceleration of the heart after small doses of nicotine. He stated that this acceleration does not occur after section of the vagi. In larger doses he admitted that nicotine diminished the excitability of the cardiac fibre, and finally destroyed its irritability. He also noted that the cardiac depression was greater in cold-blooded than in warm-blooded animals.

Langley and Dickinson⁴ have concluded from their experiments that there is no excito-motor mechanism of nerve cells in the heart. They also noted that the direct application of a 10% solution of nicotine to the heart causes some tonic contraction of the ventricle. This they however ascribe to the alkaline nature of nicotine, since they have not observed any tonic contraction when neutralized nicotine or nicotine tartrate was used. In concluding this section of their paper they write "nicotine acts on the nerve cells and not on the nerve endings of the heart⁵."

Colas⁶ believes that nicotine depresses the intracardiac nerve centres but that the acceleration is due to a direct action on the cardiac tissue. Émile Yung⁷ found that nicotine caused a great primary acceleration followed by depression in the hearts of crabs, lobsters, and Lamellibranch molluscs (i.e. solen, mya and anodon). He also observed that local application of the poison to the heart caused local arrest while the

¹ Brodie. *Phil. Trans. Roy. Soc.* Vol. c1. (1811), p. 196.

² Rosenthal. *Centralblatt f. d. med. Wissenschaft.* Hft. xlvi. S. 737.

³ Jullien. *Étude sur la Nicotine.* Paris, 1868 (with a bibliography).

⁴ Langley and Dickinson. *This Journal.* Vol. xi. (1890), p. 278 (with a bibliography).

⁵ Since Langley and Dickinson (*loc. cit.*) have shewn that physiological action of pituri is identical with that of nicotine it is interesting to note that Gibson (*Journ. Anat. and Phys.* Vol. xvi. (1882), p. 10), found that *Duboisia Hopwoodi* (a Pituri plant) first annuls the cardiac inhibitory mechanism and in larger doses stopped the heart in diastole. The actions on the cardio-inhibitory mechanisms of these drugs has been fully worked out by Langley and Dickinson.

⁶ Colas. *Comptes Rendus de la Soc. de Biol.* 9^{me} série, T. n. (1890), p. 31.

⁷ Yung. *Archives de Zool. Expérimentale.* T. vii. (1878), p. 480; *ibid.* T. ix. (1881), pp. 429-444.

remainder of the heart continued to beat. Yung's observations on the crustacean heart have been confirmed by Plateau¹.

My results with nicotine are illustrated by these experiments.

EXPERIMENT A. *Nov. 8th, 1892.* Embryo aged 81 hours. Temperature throughout the experiment 35° C. Normal frequency of heart 92 per minute. Strength of the solution of nicotine used was $\frac{1}{2}$ c.c. of Martindale's nicotine to 100 c.c. of normal saline (65% of sodium chloride) solution.

At 1.11.10 $\frac{1}{4}$ of c.c. of the above named solution of nicotine was administered.

- 1.11.30 Rhythm normal in every respect.
- 1.12.30 Rhythm normal in every respect.
- 1.16.0 Frequency 112 per minute, rhythm otherwise normal.
- 1.17.0 Frequency 112 per minute, rhythm otherwise normal.
- 1.19.0 1 c.c. of the solution of nicotine added.
- 1.20.0 Frequency 88 per minute, beats weaker than is normal.
- 1.21.0 Frequency 86 per minute, beats weaker than is normal.
- 1.24.30 Frequency 82 per minute. The systoles are very weak and the whole heart in an expanded condition.
- 1.27.0 The heart in the same condition as stated in previous record.
3 c.c. of normal saline containing .03 gram of potassium chloride was added.
- 1.28.0 Frequency 96 per minute. The systoles are strong and the diastoles are good.
- 1.29.0 Frequency 102 per minute. The systoles are strong and the diastoles are good.
- 1.30.0 Frequency 102 per minute. Same remark as above.
- 1.34.0 Frequency 110 per minute. Same remark as above.
- 1.35.0 Frequency 110 per minute and rhythm normal. *2 c.c. of above named solution of nicotine added.*
- 1.36.0 Frequency 92 per minute. Beats much weaker.
- 1.37.0 Frequency 80 per minute. Systoles very weak. Whole heart expanded.
- 1.46.0 Heart recovering frequency 98 per minute, but the systoles are still imperfect. *1 1/2 c.c. of nicotine solution administered.*
- 1.47.0 Frequency 46 per minute. Systoles very weak. Whole heart expanded.
- 1.48.0 Frequency 44 per minute. Systoles very weak. Whole heart expanded.
- 1.49.0 *4 c.c. of potassium chloride solution added containing .04 gram.*

¹ Plateau. *Archives de Biologie* (Van Beneden's). T. 1. (1880), p. 633.

1.51.0 Heart in extreme expansion with a few auricular twitches.
 1.57.0 Heart in same condition.
 1.59.0 Heart still expanded and irresponsive to stimuli.

EXPERIMENT B. *Nov. 8th, 1892.* Embryo aged 80 hours. Temperature throughout the experiment 38° C. Normal frequency at this temperature 100 per minute. Strength of nicotine solution as in above experiment.

At 12.2.15 1 c.c. of the solution of nicotine added.
 12.3.15 Frequency 74 per minute and weaker than the normal.
 12.4.30 Heart still beating but the beats are too feeble to count. It is extremely expanded.
 12.5.30 Was able to count 60 very feeble beats. The whole blastoderm has become anaemic.
 12.7.0 and 12.9.0. Beats too feeble to count. Blastoderm very anaemic.
 12.9.45 2 c.c. of potassium chloride solution added containing .02 grams.
 12.11.0 No change in condition of the heart.
 12.11.45 4 c.c. of potassium chloride solution added = .04 gram.
 12.12.45 No change observed.
 12.13.45 Heart gave a few strong beats.
 12.14.45 Frequency 80 per minute. Beats very strong.
 12.15.45 Frequency 90 per minute. Beats very strong.
 12.17.45 Frequency 90 per minute. Beats very strong.
 12.30.0 Frequency and condition same as previous record.
 12.30.45 1 c.c. of nicotine solution added.
 12.31.45 Frequency 80 per minute. Beats are weaker.
 12.32.45 Frequency 68 per minute. Beats still weaker.
 12.38.0 Frequency 60 per minute. Beats extremely feeble.
 12.39.0 6 c.c. of potassium chloride solution added = .06 gram.
 12.41.0 Frequency 56 but systoles much stronger.
 12.43.0 Frequency 60 per minute with strong systoles.
 12.45.0 Frequency and condition same as previous record.

From the above experiments it is obvious that small doses of nicotine increase the frequency of the embryonic heart, and that larger doses stop it in diastole. Nicotine therefore acts directly upon the myoplasm of the embryonic heart.

The acceleration described is very similar to the following which I quote from Langley and Dickinson's paper "After a drop of 1% nicotine has been allowed to fall upon a frog's heart, we have not observed a primary slowing to be caused by subsequent applications. Apparently the paralysed nerve cells only slowly recover. Not infrequently the

second, third, and it may be later applications of nicotine to the heart cause for 10 to 15 seconds a quickening of the heart beat, during this quickening the beats may become stronger or weaker; in the former case, the tracing obtained may be like that obtained by a brief stimulation of the sympathetic. After one or two applications, the beat of the heart is for a considerable time reduced in rate." These observers, having paralysed the inhibitory mechanism of vagus in the heart by their first application of the 1% nicotine, have by their subsequent application of nicotine obtained a stimulation of the cardiac muscle which is similar to that which I have observed on the embryonic myoplasm. A possible explanation that my primary acceleration has been of longer duration than that observed by Langley and Dickinson on the frog's heart may be found in the smaller doses given in my experiments. My observations also support the conclusions of Langley and Dickinson that there is no excito-motor mechanism in the frog's heart, since in the embryonic heart with no nervous mechanism the results tally with the frog's heart which is complicated by its nervous mechanisms. These observers have also noted in the frog's heart that the inhibitory action brought about by stimulating the sinus, disappears before the augmentor action that can be brought about by stimulating the sympathetic. This they explain by the exciting action of the electric shocks on the muscular tissue overcoming the inhibitory action of the stimulated nerves when the latter becomes weak. Another factor which may determine the phenomenon is the fact that small doses of nicotine by heightening the excitability the cardiac fibre render it more responsive to augmentor than to inhibitory stimuli.

My results are also in accord with those of Colas (loc. cit.).

There is also a remarkable correspondence in the action of nicotine on the hearts of decapodan crustaceans and lamellibranchs and that on the hearts of embryos and frogs as will be seen by the comparison of the results recorded above with those quoted from the researches of Yung and Plateau. I must however differ from Yung's¹ explanation of the expansion of the heart in nicotine poisoning which that writer believes to be owing to the contraction of the peripheral vessels. This explanation is excluded in the embryonic heart's experiments, as well as by the fact that the direct application of nicotine to the isolated frog's heart will cause diastolic dilatation. There are however great differences between the action of nicotine on the contractile mechanism of the medusæ and the contractile tissue of the heart. The primary spasms

¹ *Archives Expér. de Zool.*, T. ix. (1881), pp. 439—441.

which Romanes¹ has shewn to exist in the poisoning of these animals with nicotine are probably due to the action of the drug on the nerve plexuses which the researches of the Hertwigs² and of Prof. Schäfer³ have made evident in both *Hydromedusans* and *Scyphomedusans*. This is further evidence that nicotine "selects" nervous fibrils before it attacks contractile tissue.

We may here recall the statement previously made that potassium chloride depresses the embryonic myoplasm without any primary excitatory phase and finally stops the heart in diastole.

It is therefore remarkable that the application of potassium chloride to an embryonic heart which has been depressed almost to stoppage by nicotine should not only restore the rhythm, but raise it above the normal limit⁴. Possibly this may be explained on the chemical theory of antagonism by suggesting that the potassium chloride having antagonized a moiety of the nicotine leaves free a small quantity of that body to produce its accelerator effect by acting on the cardiac myoplasm. Larger doses of the potassium chloride though they may restore a heart depressed almost to stoppage will not produce an acceleration of rhythm above the normal frequency while in cases where the nicotine poisoning has been more complete, even larger doses of potassium chloride, though they fail to increase the frequency of the rhythm yet by improving the systoles render it more normal⁵. In still larger doses there is little doubt that the potassium chloride exerts its own depressing effect on the cardiac myoplasm.

Having obtained the antagonism of nicotine and potassium chloride and of veratrine and potassium chloride on the embryonic heart the question arose whether these antagonisms were characteristic of cardiac myoplasm (and since the latter antagonism has been demonstrated on the frog's heart) and cardiac muscle, or whether they were obtainable on contractile tissue generally.

Miss Greenwood⁶ has found that a '05 p. c. solution of nicotine produces an extension of the contractile body of *Hydra fusca* which lasts for half-an-hour, during which time the animal is irresponsive to stimuli. I have experimented with large specimens of *Hydra fusca* and can completely confirm Miss Greenwood's statements. The

¹ Romanes. *Phil. Trans. Roy. Soc.* Vol. CLXVI. (1876), p. 269.

² O. and R. Hertwig "Das Nervensystem und die Sinnesorgane der Medusen."

³ Schäfer. *Phil. Trans. Roy. Soc.*, part II. 1878.

⁴ See experiment A. ⁵ See experiment B.

⁶ Greenwood. *This Journal.* Vol. XI. (1890), p. 582.

addition of potassium chloride in all strengths does not restore the irritability of the animal although in some cases it changed from an expanded to a contracted condition after the application of the potassium chloride. In other cases no such change was observed. Potassium chloride in amounts of '002 gram in 2 c.c. of water caused hydras to pass into a contracted condition in which they were irresponsible to stimuli. Nicotine failed to restore the animals. Doses of '0015 gram of potassium chloride are apparently innocuous. Veratrine given in doses of '002 gram in 2 c.c. of water also caused hydras to pass into a contracted or semi-contracted condition in which they were irresponsible to stimuli. I have failed to restore their irritability by the application of potassium chloride.

As pointed out by Langley and Dickinson¹ the direct application of a 1 p. c. solution of nicotine to a skeletal muscle causes an increase of tone—a slight contraction of the muscle—as well as fibrillar twitchings. I have increased the dose up to a 4% solution of nicotine when the muscle becomes still more contracted and responds but feebly to electric stimulation. I have failed to restore the muscle's power of response to stimuli by the application of potassium chloride. It therefore seems probable that the antagonisms described above are characteristic of cardiac myoplasm and cardiac muscle².

On Physiological Antagonism.

We are now in a position to make a few remarks on the chemical theory of antagonism, as far as it occurs, in action of drugs on cardiac tissues. The chemical theory of antagonism must rest upon the view that the stimulation of contractile tissues depends on a chemical combination between the acting substance and the contractile material, with a resulting new substance, of different chemical constitution but still possessing the power of contraction. That these antagonisms of drugs occur there is no doubt, the older objections of Rossbach³ having been answered by Langley⁴ and others⁵. As regards cardiac tissue it

¹ Langley and Dickinson. *Op. cit.* p. 274.

² I may however remark that Brunton and Cash (Brunton's *Pharmacology*, 3rd ed. p. 493), have antagonized salts of potassium and calcium in skeletal muscles of the frog.

³ Rossbach. *Pflüger's Archiv.* Bd. xxi. (1879), Hft. 1.

⁴ Langley. *Journ. Anat. and Physiol.* Vol. x. (1875). *Ibid.* Vol. xi. p. 173. This *Journal*. Vol. i. (1878), p. 320. *Ibid.* Vol. iii. (1880), p. 11.

⁵ Vide Ringer and Morshead. This *Journal*. Vol. ii. p. 235. Ringer. This *Journal*. Vol. v. p. 246.

has been urged by Schmiedeberg¹ that alteration in the elasticity of the contractile tissue may without chemical combination be the cause of the change of rhythm. Reference back to the *Hydra* experiments described above will shew that a change from expansion to contraction may take place in a contractile tissue, while there is no restoration of the power of response to stimuli. It is therefore possible that the molecular changes induced by an acting drug may be accompanied by changes in the elasticity of the tissue.

Loew² has recently shewn that caffeine combines with certain portions of protoplasmic cells and caffeine is known to exert definite action on contractile tissue. In a previous paper I have shewn that egg-albumen will combine with salts of cobalt, and that salts of nickel will displace salts of cobalt from the proteid molecule and these again will be displaced by salts of copper. In this case we have three metals mutually displacing each other by combining with the proteid molecule.

There is therefore chemical evidence that certain substances can loosely combine with the proteid molecule and be turned out of their combination by other substances which displace them and this lends support to the chemical theory of antagonism.

Further it has been argued that the amount of the drug present in certain cases of physiological antagonism is too small for chemical combination. In the cobalt, nickel and copper displacements the amount of the metal used is almost as small as the amount of the drug used in physiological antagonism. Yet the whole nature of the proteid molecule is changed as evidenced by its colour reactions. It is also to be noted that both potassium salts and nicotine as well as veratrine stop the heart in diastole, yet the application of potassium salts tends to improve the systoles of a heart that has passed into expansion either due to nicotine or to potassium chloride.

There is therefore evidence that the elasticity of contractile tissues plays only a secondary part in the mechanism of their response to the antagonistic action of drugs.

Hydrocyanic Acid.

The main features of the action of this body on the adult mammalian heart have long been known owing to the researches of Preyer³, who found that the ventricular beats became very irregular,

¹ Schmiedeberg. *Ludwig's Festgabe*, 1874.

² Loew. *Op. cit.* p. 32.

³ Preyer. *Virchow's Archiv.* Bd. XL. (1867); S. 125.

the contractions at one time being strong while at others were mere twitches, arrest was in extreme diastole. He also emphasised the fact that the hydrocyanic acid combined with blood.

My results with the embryonic heart are illustrated by the following experiment.

Nov. 10th, 1892. Embryo aged 73 hours. Normal rhythm 112 per minute. Temperature throughout the experiment 30° C.

At 11.32.30 1 e.e.¹ of hydrocyanic solution in normal saline given.

11.33.30 Frequency 80 per minute. Systoles apparently much stronger than is normal. Diastoles good.

11.34.30 Frequency 60 per minute. Systoles very strong. Diastoles weak.

11.36.30 Frequency 50. Both diastoles and systoles very strong. The rhythm is very regular.

11.39.0 Frequency and remarks as above. A second e.e. of the *hydrocyanic acid solution added*.

11.40.0 *Reversed rhythm* of 40 per minute. Both systoles and diastoles complete.

11.41.0 Frequency 34 per minute. The rhythm is apparently almost simultaneous. I was unable to determine from which end the contraction wave originated.

11.47.0 Frequency 32 per minute, *normal in direction*. The diastoles are perfect but *systoles are weak*.

11.51.0 The rhythm *reversed in direction but regular*. The systoles are weak.

11.56.0 Frequency 30 per minute and regular. The systoles are sometimes perfect. At others imperfect. The diastoles are constantly good.

12.1.0 1 c.c. of solution of hydrocyanic acid added.

12.2.45 Frequency 36 per minute. Both systoles and diastoles good.

12.3.45 Ventricular frequency 34 per minute, auricles however are beating at a higher frequency.

12.4.45 Ventricular frequency 20 per minute. About one in every four of the auricular beats pass to the ventricle.

12.16.0 Ventricle stopped in extreme expansion. The auricles expanded, and gave four twitches during the minute of the observation.

12.17.0 A mechanical stimulus started the whole heart with a rhythm of 68 per minute.

¹ The strength of the solution used was 10 c.c. of hydrocyanic acid of the British Pharmacopœa to 400 c.c. of normal saline.

12.18.0 The whole heart stopped in diastole.

12.19.0 Mechanical stimulus applied to the ventricles caused two beats to commence from the auricular end. The heart then stopped in diastole.

12.22.0 Stimulus applied to ventricle resulted in fifty-two beats which commenced from the auricular end. Whole heart stopped in diastole.

12.25.0 Mechanical stimulus to ventricle resulted in two beats commencing from the auricles.

12.26.0 Similar stimulus resulted in 42 beats, commencing from the auricles. Heart finally stopped in diastole.

12.27.0 The expanded heart fails to respond to mechanical stimuli applied to the ventricle.

12.28.0 Heart though failing to respond to ventricular stimuli, after three touches with a needle of auricles, gives seven complete beats.

12.30.0 Did not respond to either auricular or to ventricular stimulation.

12.32.0 Failed to respond to repeated ventricular stimuli, but on repeated touching of the auricle with the needle's point, it gave fourteen feeble beats which spread to the ventricle. The heart then stopped in extreme diastole.

12.42.0 Auricular stimulation by needle point caused four feeble beats which did *not* spread to the ventricle.

12.47.0 Heart irresponsive to stimuli if applied either to auricle or ventricle. It died in extreme diastole.

The experiment recorded above seems to point to a complex action of the hydrocyanic acid. The first action of small doses is to depress the frequency of the rhythm but at the same time to apparently improve the systoles, while the diastoles are apparently weaker than in the normal rhythm. As emphasised by Preyer¹ hydrocyanic acid combines with blood—it is therefore not improbable that the first action is not due to the direct action of the hydrocyanic acid upon the embryonic myoplasm but to an indirect action as a cyanogen compound of haemoglobin. The second application of the hydrocyanic acid results in a reversal of the rhythm before impairment of the systoles has set in. The reversal of rhythm will be discussed at greater length in a subsequent paragraph but we may here note, that in the case of the action of hydrocyanic acid and of amyl nitrite it is probable that the direct action on cardiac myoplasm is mixed with the indirect action,

¹ Preyer. *Op. cit.*

in the first case of the cyanogen haemoglobin compound and in the second case of the methaemoglobin. Morphine is another drug which produces a reversal of the rhythm of the embryonic heart.

Morphine has been shewn by Brunton and Cash¹ to be a powerful depressant to the oxidizing power of protoplasm. In methaemoglobin the oxygen is in more stable combination with the haemoglobin molecule than in the oxyhaemoglobin molecule and consequently the oxidization processes of the tissues or cells that are supplied with either methaemoglobin or cyano-haemoglobin instead of oxyhaemoglobin must be diminished. It therefore seems that there is some relation between the reversal of rhythm and the suspension or interference with the normal processes of oxidation in the embryonic myoplasm. Further it may be urged as negative evidence that the drugs caffeine and digitalin which produce profound changes in the cardiac myoplasm do not lead to a reversal of rhythm and these substances have been shewn by Brunton and Cash² to improve the oxidizing power of protoplasm.

The second phase of the action of the hydrocyanic acid agrees with the condition discovered by Gaskell³ as being characteristic of the action of lactic acid on heart muscle. The condition of expansion which is induced is probably due to the direct action of the hydrocyanic on the embryonic myoplasm, and corresponds to the expansion produced by the action of dilute acid solution (*e.g.* lactic and acetic) on the embryonic myoplasm. In this second phase of action the auricular rhythm is dominant over the ventricular rhythm, the ventricles being in a condition of greater expansion than the auricles, thus we note that the auricular frequency is greater than the ventricular frequency, some of the beats are blocked at the auriculo-ventricular junction. When this phase passes into its extreme condition we note that ventricular stimulation leads the commencement of beats from the auricular end, that is, in the normal direction. It is thus probable that the generic action of hydrocyanic acid, as an acid, obscures when given in larger doses direct to the embryonic heart the specific action which it exhibits owing to its cyanogen radicle. The fact that hydrocyanic acid stops the adult heart in diastole would seem to shew that its specific action also stops the heart in diastole, since the doses given could not have been sufficient to change the reaction of the blood.

¹ Brunton and Cash. *St Barts. Hosptl. Reports*, 1882.

² Brunton and Cash. *Op. cit.*

³ Gaskell. *This Journal*. Vol. III. p. 56.

Romanes¹ found that potassium cyanide first quickened and then enfeebled the contractile functions of medusæ, the animal finally passed into a contracted condition. We here note that there is a marked difference in this action to those of hydrocyanic on the embryonic myoplasm, and whether this difference is due to the potassium in the molecule or to a fundamental difference in the two types of contractile tissue I hope to answer by a future series of experiments.

To summarize my results with hydrocyanic acid I state that:—The action of hydrocyanic acid is complex, the first phase, being due to that of the cyanhaemoglobin formed and possible to the depression of the oxidation processes resulting from the substitution of cyanhaemoglobin for the normal oxyhaemoglobin, the second phase is probably due to the action of hydrocyanic acid generically as an acid and resembles the well known action of lactic acid on cardiac muscle.

Nitrite of Amyl.

B. W. Richardson² found that amyl nitrite caused dilatation of the blood vessels in the web of the frog's foot. Also after absorption it caused increase of cardiac frequency followed by diminished action. Brunton³ found that the number of heart-beats was especially increased in dogs. He however inclined to the view that its chief action was via the capillaries.

H. C. Wood⁴ confirmed many of Brunton's results, and believed that the reduction of heart frequency was due to direct action on the cardiac muscle. By local application to the heart he obtained progressive diminution of the rhythmic power without a primary phase of acceleration.

Amez-Droz⁵ also found acceleration of the heart and extreme capillary dilatation.

My results are as follows.

With small doses produced by evaporation of amyl nitrite within on the incubator so that the atmosphere around the blastoderm is charged with the

¹ Romanes. "Jelly Star-fish and Sea-Urchins," p. 232. *Internat. Science Series*, Vol. L.

² Richardson. *Brit. Assoc.* 1864. *Reports* 1865, p. 280.

³ Brunton. *Journ. Anat. and Physiol.* Vol. v. *Bericht d. mathem.-phys. Classe d. königl. Sächs. Gesellschaft der Wissenschaft*, 1869. *Lancet*, 1867. Vol. II. p. 97.

⁴ Wood. *Amer. Jour. Med. Sci.* Vol. LXII. (1871), p. 39.

⁵ Amez-Droz. *Archives de Physiol.* Vol. v. (1873), p. 467.

vapour there is a primary acceleration of the embryonic heart which is however not well marked. In an embryo beating with a rhythm 96 per minute its frequency rose after three minutes' action to 102 per minute which was maintained for four minutes, when the rhythm returned to the normal. After ten minutes' longer action there was a depression of the rhythm to 90 per minute.

When given in larger doses dissolved in olive oil direct on the embryonic heart (the strength being $\frac{1}{2}$ c.c. of amyl nitrite to 100 c.c. of olive oil (dose = $\frac{1}{4}$ c.c. of this solution) there was a depression of the rhythm from 124 beats per minute the normal to 112 beats per minute. Further applications of $\frac{1}{2}$ c.c. of this solution reduced to rhythm 100 per minute and after 1 c.c. of this solution had been given the frequency was reduced to 50 per minute and the rhythm reversed. The rhythm remained reversed for several minutes, and the heart finally stopped in diastole.

The action of amyl nitrite is interesting because it probably depends on the nitro group (NO_2). It is also probably complex, part resulting from the methæmaglobin formed and part from its own specific action. The oxygen in the methæmaglobin being more firmly combined than in normal oxyhæmaglobin there is probably a reduction of the oxidation processes in the embryonic myoplasm. The relation of this action to the reversal of rhythm and diastolic stoppage will be considered in other sections. The appearance of Luciani's beats just before the death of an embryonic heart poisoned by amyl nitrite favours the view that its oxidation processes are diminished, since this abnormality of cardiac action characterizes hearts deprived of oxygen.

Morphine Acetate.

My results with morphine acetate are illustrated by the following experiments.

A. Dec. 10, 1892. Embryo aged 80 hours. Temperature throughout experiment 40°C . Normal frequency 140 per minute.

At 2.58.0 1 c.c. of normal saline containing '0002 gr. of morphine acetate was given.

- 2.59.0 Frequency 110 per minute. Beats irregular.
- 3.0.45 Frequency 102 per minute. Beats irregular with frequent stoppages. Rhythm reversed that ventricles beat before auricles.
- 3.2.25 Frequency 102. Beats sometimes normal in direction, at others reversed.
- 3.6.0 Frequency 106. Beats irregular, sometimes normal in direction, at others reversed.

3.8.0 1 c.c. containing '0002 gr. of morphine acetate given.

3.13.0 Rhythm during last five minutes 74 per minute, and irregular.

3.15.0 '0002 gr. of morphine acetate given.

0.17.0 Rhythm very irregular, there being 86 beats in the minute, sometimes it is very rapid, at others reversed while it may pass almost into diastolic stoppage.

3.19.0 Heart in same condition.

3.20.0 Frequency 112 during the minute but the rhythm extremely irregular, shewing same phenomena as recorded above.

3.23.0 Heart in same condition. '0002 gr. of morphine acetate given.

3.24.0 76 beats during the minute. General condition unchanged.

3.28.0 '0004 gr. of morphine acetate given.

3.31.0 68 beats during the minute. General condition unchanged.

3.33.0 '0004 gr. of morphine acetate was given.

3.35.0 Frequency 60 per minute. Conditions unchanged.

3.40.0 Rhythm during last five minutes extremely irregular, and averaging 54 beats per minute.

Further doses produced similar results to those recorded above with final death in diastole.

B. Dec. 12, 1892. Embryo aged 70 hours at a temperature of 31° had a dose of '0001 of morphine acetate. It reduced its rhythm from 134 beats per minute to 96 beats per minute, and there were no irregularities. On rapidly raising the temperature of the heart to 40° C. the rhythm became 156 beats per minute. Further doses of morphine acetate at the higher temperature gave results similar to those recorded in experiment A.

It is therefore evident that while morphine acetate when given at comparatively low temperatures to the embryonic heart is a simple depressant at high temperatures besides the slowing of the rhythm, marked irregularities, reversal of rhythm together with periods of rest alternating with violent bouts of rapid beating characterize its action. In both cases death is always in diastole.

The action of morphine acetate at the lower temperature is comparable to its simple depressant action described by Romanes¹ on medusæ.

On the adult heart Blyth² thus describes its action "The beats are first accelerated then diminished in frequency; but very large doses introduced directly into the circulation at once diminish the pulsations

¹ Romanes. *Op. cit.*

² Blyth. *Poisons: Their effects and detection*, 1884, p. 284.

and no acceleration is noticed. The slowing is central in its origin, for on the vagi being cut morphine always quickens."

Ludwig¹ introduced tincture of opium into the mammalian ventricle with the result that the rhythm of the heart was reversed. The phenomena described as characterizing the embryonic heart under the influence of morphine acetate at higher temperatures are thus similar to those occurring in the hearts of warm-blooded animals. It will be noticed that a rapid increase of temperature will abolish the action of the drug (Exp. B) on the heart causing it to beat with a rhythm of greater frequency corresponding to the higher temperature.

It has been shewn by Harley² that morphine lessened the processes of oxidation in the blood. Reference to the section on hydrocyanic acid will shew the reader that the formation of cyanhæmoglobin by that substance, and also the formation of methæmoglobin (see p. 425) by the action of amyl nitrite on the blood of the embryo must diminish the oxidizing power of that blood. Now in the cases where the oxidizing power either of the blood or of the contractile substance of the embryonic heart is diminished we note an atonic condition and reversal of rhythm. Also it is well known that heat favours oxidation processes and heat will remove the atonic condition and restore the normal sequence of rhythm in a morphinized heart. It is also possible that many of the differences that have been recorded between the batrachian heart and that of mammals are owing to the difference of temperature at which their respective metabolisms go on.

On Reversed Rhythm.

The reversal of rhythm which seems to characterize certain phases in many forms of heart poisoning in the embryo has been observed under a variety of conditions in different hearts. Thus its normal occurrence was discovered long ago by Van Hasselt⁽¹⁾ and Eschscholtz⁽²⁾ in the Urochorda and according to Fol⁽³⁾ in many appendicularians. Gaskell⁽⁴⁾ has shewn that it is easily excited by mechanical stimulation in many Selachian hearts, while McWilliam⁽⁵⁾ has induced it either by mechanical stimulation or by the application of a constant electric current in the eel's heart. The same observer noted it after profound vagus inhibition in the same animal. Wesley-Mills⁽⁶⁾ obtained it by mechanical stimulation of the ventricle of the tortoise. McWilliam⁽⁷⁾ extended his observations to the mammalian ventricle and found that a

¹ Ludwig. *Lehrbuch der Physiologie des Menschen.* Bd. II. (1861), S. 88.

² Harley. *Phil. Trans.* 1865, p. 678.

single induction shock applied to the ventricle during diastole induced a single reversed beat and in very excitable ventricles a mechanical, thermal, or even a single electrical stimulus may induce a series of reversed beats and in any heart, a reversed rhythm may be kept up by applying to the ventricle a series of single stimulations of somewhat greater rapidity than the normal spontaneous rhythm. Reversed rhythm is also very easily induced during a cardiac standstill, produced by stimulation of the vagus, a single excitation of the ventricles giving a single reversed beat, while a series of ventricular excitations gave a series of reversed beats. Also reversed contractions sometimes occur spontaneously at certain phases of vagus influence. Further in hearts of dying kittens and in the hearts of cats poisoned by potassium bromide the application of a stimulus to the ventricle, or the exaltation of the excitability of the cardiac tissue by the local application of heat will originate a wave contraction from the point of application of the stimulus.

Von Bezold⁽⁸⁾ found that when a frog's heart has been brought to a standstill by the performance of Stannius' experiment, any stimulus if applied to the ventricle will originate a reversed rhythm which will often persist for a considerable time. Bernstein⁽⁹⁾ also found that if a constant current be applied to heart muscle under the same conditions, rhythmic contractions in the direction of the current which are reversed by reversing the current result. This was confirmed by Foster and Dew-Smith⁽¹⁰⁾ who also have shewn a similar reversal of rhythm in the snail's heart⁽¹¹⁾ when acted upon by a constant current. Ludwig⁽¹²⁾ noted a reversal of rhythm as the result of mechanical stimulation of a mammalian ventricle that had been stopped by opium poisoning; while Langley and Dickinson⁽¹³⁾ found in frogs' hearts which had been given a small dose of nicotine and inhibited by stimulation of the sino-auricular line that a touch on the ventricle usually causes a single reversed beat.

May not a possible explanation of these phenomena as well as those exhibited by the embryonic heart, be found in the fact that the contractile substance of both auricles and ventricles is endowed with automatism, and that when owing to external circumstances of a depressing nature whether electrical or chemical the functional activity of the auricular contractile substance is lowered below that of the ventricular contractile substance then a ventricular stimulus which would normally be conducted to the auricle is blocked by the debilitated condition of the auricular substance and acting locally originates a contraction wave which starts from the

point of stimulation? In support of this hypothesis may be urged the fact that conductivity is destroyed before the power of local response of stimulation. In the case of the spontaneous reversal of rhythm in the embryonic heart under the influence of certain drugs, a greater depression of the auricular myoplasm than of the ventricular myoplasm would cause the automatism of the ventricle to overcome that of the auricle and a reversal of the rhythm to take place. If the view put forward on p. 423 that the reversal of rhythm is in some degree related with diminished or impaired oxidation processes, be approximate to truth, it would then seem that the auricular myoplasm of the embryo and auricular muscle of the adult was more dependent on ample oxidation and consequently more depressed by lack of oxygen than the ventricular myoplasm or muscle.

This view is in accord with the greater activity of the auricular contractile substance, since greater activity is usually associated with more rapid processes of oxidation.

Another class of reversed rhythm is that which is liable to occur during vagus stimulation, the most marked being the spontaneous reversal described by McWilliam⁽⁵⁾ on the mammalian ventricle. Roy and Adami¹ have also in the mammalian ventricle shewn that vagus stimulation will produce an independent ventricular rhythm and thus that the vagus depression of the auricular contractile substance is greater than vagus depression of the ventricular contractile tissue. It would therefore seem that the same explanation will account for the reversal of rhythm in certain phases of vagus action, as in certain phases of toxic action viz. that when the depression of the auricular contractile substance reaches a certain intensity, then the automatism of the ventricle becomes dominant and the rhythm is reversed.

LIST OF PAPERS DEALING WITH REVERSED RHYTHM.

- (1) Van Hasselt. *Ann. des Sciences Nat.* T. III. (1824), p. 78.
- (2) Eschscholtz. *Bericht. über die zoologische Ausbeute der Reise von Kronstadt bis S. Peter und S. Paul.* (1825), Bd. xv. S. 738.
- (3) Fol. *Études sur les Appendiculaires.* 1872.
- (4) Gaskell. *Jour. Phys.* Vol. iv. p. 78.
- (5) McWilliam. *Proc. Roy. Soc.* Vol. XXXVIII. p. 108. *Jour. Phys.* Vol. VI. p. 192. *Proc. Physiolog. Soc.* Dec. 13th, 1883.
- (6) Wesley-Mills. *Jour. Phys.* Vol. VII. (1886), p. 81.
- (7) McWilliam. *Jour. Phys.* Vol. IX. (1889), p. 178--186.

¹ Roy and Adami. *Phil. Trans.* Vol. 183 (1892), p. 199 to 298.

- (8) von Bezold. *Virchow's Archiv.* Bd. xiv. S. 282.
- (9) Bernstein. *Nerv. u. Muskel.* Abschnitt v. S. 205.
- (10) Foster and Dew-Smith. *Jour. Anat. and Phys.* Vol. x. p. 735.
- (11) Foster and Dew-Smith. *Proc. Roy. Soc.* Vol. xxiii. p. 318.
- (12) Ludwig. *Lehrbuch der Physiologie.* (1861), Bd. ii. S. 88.
- (13) Langley and Dickinson. *Jour. Phys.* Vol. xi. (1890), p. 279.

Potassium Chloride.

With potassium chloride I obtained results that can be illustrated by the following experiment.

Oct. 12th, 1892. Embryo aged 72 hours. Temperature throughout the experiment 30° C. Normal rhythm 76 per minute.

At 2.17.45 $\frac{1}{2}$ c.c. of normal saline solution was added containing '005 gram of potassium chloride.

- 2.19.10 Frequency 64 per minute. Beats very regular and strong.
- 2.20.15 Frequency 68 per minute. Beats unchanged.
- 2.21.0 Same as preceding observation.
- 2.26.0 No variation in the rhythm during the past five minutes. '02 gram of potassium chloride was given.
- 2.27.0 Frequency 64 per minute. Rhythm otherwise unchanged.
- 2.32.0 '02 gram of potassium chloride was given.
- 2.34.0 Frequency 50 per minute. Systoles were weaker than is normal.
- 2.35.0 '02 gr. of potassium chloride was given.
- 2.36.0 Heart stopped in diastole.
- 2.40.0 Expanded heart irresponsive to either mechanical or electrical stimuli.

All attempts to antagonise its action with veratrine failed.

The above experiment shews that potassium chloride acts upon the embryonic myoplasm depressing its functions by weakening the systolic power, and finally stops the heart in diastole. It is interesting to note that veratrine stops the heart by lengthening out the systoles, and the heart stopped by veratrine is like the heart stopped by potassium chloride in a state of extreme expansion. Notwithstanding this apparent similarity of action, veratrine is as will be pointed out in a subsequent section antagonized by potassium chloride both in its action upon the hearts of frogs and embryos (see p. 442). This fact strongly militates against the view that alterations of extensibility play an important part in the antagonism of drugs acting upon the cardiac

contractile substance, since both veratrine and potassium chloride tend to expand the embryonic myoplasm.

The failure to antagonize potassium chloride by the action of veratrine may be explained by Langley's¹ statement "A limit is placed to (this²) antagonism by the impossibility of giving very large quantities of any substance without injuring the tissue by physical processes consequent on the alteration in the density of the fluids. The antagonistic action of alkaloids can only occur within the limits of doses which do not seriously alter the tissue by altering the normal rate of diffusion &c." It is therefore probable that potassium chloride has a greater affinity for combination with the embryonic myoplasm than veratrine has. The paralysing action of potassium chloride on the embryonic myoplasm agrees closely with that described long ago by Blake³ on the frog's heart. A more complete knowledge of its action on that animal is due to Ringer⁴, who found that potassium chloride reduced gradually the ventricular contractions until the heart stopped in diastole. It is interesting to compare these results with those of Brunton and Cash⁵ who experimenting on the action of potassium chloride on blood vessels found that it caused great contraction. It would therefore seem that the action described by Brunton and Cash was either associated with a nervous mechanism, or that there is a fundamental difference between the unstriated fibres of the arterial wall and the contractile substance of the embryonic and adult heart. That such a difference may exist is probable, since Bradford and Phillips⁶ have shewn that strophanthin exerts little or no constrictor action on the renal vessels, while its tonic action on the ventricular muscle is well known. This points to a marked physiological difference between the unstriated muscle of the arterial wall and the more highly differentiated contractile substance of the heart; and it is an instance of how the selective action of drugs may differentiate the functions of tissues.

Wernieke and Preyer noted that potassium nitrate was extremely toxic to the embryonic heart, while the nitrates of sodium and ammonium in comparable doses were innocuous. Reference back to the preceding experiment will shew that doses of .005 gram of potassium chloride

¹ Langley. *This Journal.* Vol. III. p. 19.

² The antagonism under consideration was atropin and pilocarpine.

³ Blake. *Archives Générales de Médecine*, (Paris). T. vi. (1839), p. 289.

⁴ Ringer. *Practitioner*. Vol. xxviii. (1882), p. 1.

⁵ Brunton and Cash. See Brunton's *Pharmacology*, (3rd ed. 1891), p. 281.

⁶ Bradford and Phillips. *This Journal*. Vol. viii. (1887), p. 122.

diminished the frequency of the embryonic heart; now 1 c.c. of normal saline solution contains .0065 gram of sodium chloride, yet an indefinite number of cubic centimetres of normal saline can bathe the blastoderm and embryonic heart without influencing the rhythm, if the other conditions of the experiment remain unaltered. This shews that potassium salts are far more toxic to the embryonic myoplasm than sodium salts and is in agreement with the known relative action of sodium and potassium salts on other tissues.

Barium Chloride.

My results are illustrated by the following experiment.

Dec. 5th, 1892. Embryo aged 75 hours. Temperature at the commencement of the experiment 36°C . Frequency of heart-beat at this temperature 110 per minute.

At 12.10.30 1 c.c. of normal saline containing .0002 gram of *barium chloride* was given.

12.21.0 Rhythm during last ten minutes remained apparently unchanged.

12.21.10 .0002 gram of *barium chloride* given.

12.26.0 Frequency during last three minutes 88 per minute. No apparent change in the character of the rhythm.

12.40.0 Temperature had been allowed to fall gradually to 31.8°C ., and the frequency of the rhythm had gradually risen until it attained 140 beats per minute.

12.56.0 Temperature had fallen to 29.9° and rhythm remained at 140 per minute.

1.0.0 Temperature 29.9° . Frequency 130 per minute.

1.9.0 Temperature raised to 33°C . Frequency fell to 120 per minute.

1.12.0 Temperature 35°C . Frequency 120 per minute.

1.15.0 Temperature rapidly raised to 39°C . Frequency 140 per minute. Rhythm shews no abnormal characteristics.

1.46.0 Temperature slowly raised to 42°C . Rhythm during last ten minutes was 146 per minute and shewed no abnormal characteristics.

1.47.0 1 c.c. of normal saline containing .0004 gram of *barium chloride* given.

1.51.0 Rhythm regular at 140 per minute.

2.0.0 Temperature allowed to fall to 39°C . Frequency 130 per minute.

2.7.0 Temperature 33° C., and slowly falling frequency during last three minutes 110 per minute.

2.9.0 Temperature fell to 32° C. Frequency 110 per minute.

2.14.0 Temperature had *fallen* to 29.8° C. Frequency *rose* to 120 per minute.

2.17.0 Frequency 120 per minute; temperature 29.6° C.

2.20.0 Frequency 120 per minute; temperature 29.5° C.

2.26.0 Frequency 84 per minute; temperature 25° C.

2.31.0 Frequency 70 per minute; temperature 25° C.

In larger doses barium chloride acts as a simple depressant and finally stops the embryonic heart in diastole, thus giving results like those obtained by Blake¹ and by Ringer on the frog's heart. I have however failed to antagonise the action of barium chloride on the embryonic heart by potassium chloride, even when the depression caused by the barium chloride was not very great. I shall however make further experiments on this point since barium chloride has obtained additional interest owing to its relationship to rigor mortis and blood coagulation. The effect of variations of temperature on the action of small doses of barium chloride seems to shew that the depressant action of small doses of that substance is more marked on the embryonic heart at temperatures near the normal, than at temperatures slightly above or below the normal. Extremes of temperature however produce their characteristic effect. It is interesting to compare this result with that of Ringer² who found that veratrine had a more powerful action on the frog's heart in warm weather than at lower temperatures.

On antagonism in its relation to the theories of muscular contraction.

Some years ago Hermann³ laid stress on the similarity between the contraction of living muscle and rigor mortis and even went as far as to believe that the two phenomena were comparable, and that an incipient rigor mortis occurs at each contraction of a living muscle. The phenomena of idiomuscular contraction were regarded by this observer as intermediate between normal contraction and rigor mortis.

Additional interest was added to this theory when Halliburton⁴

¹ Blake. *Archives Générales de Médecine*. Paris, 1839. T. vi. p. 289.

² Ringer. *This Journal*. Vol. v. p. 353.

³ Hermann. *Elements of Physiology* (translated by Gamgee, 2nd ed. 1878), p. 297. Contains a resumé of Hermann's arguments.

⁴ Halliburton. *Text-Book of Chemical Physiology*, p. 408 et seq.

found myosinogen and myosin were mutually convertible, the one into the other. Halliburton thus writes "it is therefore quite possible that during contraction the proteid myosinogen may undergo certain intramolecular rearrangements, perhaps of the same nature as those which occur in a far greater degree on the death of the muscle, in each case leading to the liberation of acid. But with regard to the formation of a clot during contraction, there is one physical change which in particular shews there is a great distinction between dead muscle and contracted living muscle. This change is the alteration in the extensibility of the muscle which in rigor mortis is diminished and in contraction is increased." In connection with this point it is necessary to reinvestigate the extensibility of (1) muscle in idiomuscular contraction, (2) of cardiac muscle in tonic contraction induced by certain drugs, like caffeine &c.

Ringer¹ has described on the frog's ventricle the reciprocal antagonism between calcium chloride and potassium chloride, and Brunton and Cash² between barium chloride and calcium chloride on the one hand and potassium chloride on the other in their action upon skeletal muscle. Recently Arthus and Pagès³ have shewn that blood plasma can be prevented from coagulating by decalcifying it by mixing it with a solution of 0.1 per cent. of potassium oxalate. Coagulation sets in on the addition of either calcium, barium, or strontium chlorides. There is therefore a complete antagonism between potassium oxalate and the alkaline earths in their relation to blood coagulation. Ringer⁴ has shewn that calcium salts play the same rôle in rigor mortis that they do in blood coagulation, and further he has shewn that while calcium phosphate causes persistent ventricular spasm when applied to the heart, a mixture of potassium chloride and calcium phosphate constitutes an efficient circulating fluid and that veratrine will to some extent replace calcium salts in a circulating fluid⁵.

There is thus a striking similarity between the antagonism of calcium and potassium salts⁶ in the changes of

¹ Ringer. *This Journal*. Vol. v. p. 353.

² Brunton and Cash. *Brunton's Pharmacology*, p. 493.

³ Arthus and Pagès. *Archives de Physiologie*, 1890.

⁴ Ringer. *This Journal*. Vol. viii. (1887), p. 15.

⁵ Experiments will be made to see if veratrine will replace calcium salts in blood coagulation.

⁶ For certain recent work on coagulation see the author's paper. *This Journal*, Vol. xiv. (1893).

coagulation; and their antagonism in their pharmacological action on the contractile substance of the heart.

Without forming any premature conclusion it is well to remember these relations, in their bearing on the statements quoted above from Hermann and Halliburton.

On the Tone of the Embryonic Heart.

That the embryonic heart has a condition of normal tone is shewn by the fact that variations of temperature as well as the administration of certain substances change the volume of the heart while it continues its rhythmic beat. Thus caffeine, digitalin, and strophanthin as well as a hypernormal temperature reduce the volume of the living heart, while chloroform, strychnine, morphine, nicotine and a subnormal temperature reduce the completeness of the systoles. Since Gaskell¹ has shewn that small arteries are brought into an atonic (*i.e.* dilated) condition by acids and into a tonic (constricted) condition by alkalis, I propose to discuss together the conditions of tonus of the heart and small arteries. On this subject Foster² writes, "It has been supposed that this intrinsic tone is dependent on some local nervous mechanism, in the ear at least no such mechanism has yet been found and indeed no such peripheral nervous mechanism is really necessary."

The existence of a normal tone in the embryonic heart strongly favours the view quoted above. Those drugs which cause a tonic contraction of the embryonic myoplasm, likewise produce a tonic contraction of the adult heart, while those drugs which produce an atonic state of the embryonic heart also produce an atonic state of the adult heart. From this I conclude that the phenomena are in the two cases strictly comparable.

It is probable that the tone of an organ depends on the metabolic condition of that organ and consequently drugs and temperature variations which alter the metabolism of an organ vary its tone.

Klug³ shewed that oxygen favoured the cardiac pulsations in the adult, Fano (*op. cit.*) in the embryonic heart.

Carbonic acid according to the same observers depresses the heart rhythm in embryo and adult.

¹ Gaskell. *This Journal.* Vol. III. p. 65.

² Foster. *Text-Book of Physiol.* 5th ed. part I. p. 329.

³ Klug. *Archiv Anat. u. Phys.* (Phys. Abth.), 1879, S. 435.

Adamkiewicz¹ believes that the alkalinity of the tissue fluids aids the oxidation processes throughout the body, and alkalis have been shewn to produce to the tonic state.

Caffeine and digitalin according to Brunton and Cash² hasten the oxidation of protoplasm; caffeine and digitalin are par excellence the drugs that induce the tonic condition.

Conversely according to the same observers strychnine, morphine, nicotine, and veratrine, retard the oxidizing power of protoplasm and these are the drugs which produce an atonic state. These facts³ together with evidence adduced by Gaskell renders it probable that there is some connection between the tonic condition and the oxidation processes of the tissue in that state.

It may also be noted that Harley has shown (*loc. cit.*) that alcohol, morphine, quinine, nicotine, and strychnine all diminish the oxidation processes of the blood, and that all these substances tend to produce an atonic condition of the heart.

Ammonia.

The following experiment illustrates the action of ammonium hydrate.

Nov. 28th, 1892. Embryo aged 70 hours. Temperature throughout the first part of the experiment 38° C. Normal frequency 120 per minute.

At 10.50.0 1 c.c. of normal saline containing '00075 of a c.c. of concentrated liquor ammonie.

- 10.55.0 The rhythm during the past five minutes remained normal. Another c.c. was given containing '00075 c.c. of ammonia.
- 11.6.0 The four observations during the last eleven minutes shewed no change in the rhythm.
- 11.7.0 1 c.c. of normal saline containing '001 c.c. of ammonia was given.
- 11.9.0 Frequency 116 per minute. The systoles seem more complete than in the normal condition.
- 11.15.0 Frequency 116 per minute. No noticeable variation in the characters of the rhythm from the normal.
- 11.16.0 1 c.c. containing '001 c.c. of ammonia was given.
- 11.17.0 Frequency 114 per minute. The systoles were apparently more powerful than in the normal condition.

¹ Adamkiewicz. *Archiv Anat. u. Phys.* 1879, S. 370.

² Brunton and Cash. *St Bartholomew's Hospitl. Reports*, 1882.

³ See also sections on morphine, hydrocyanic acid and amyl nitrite in this paper.

1.22.0 Frequency 130 per minute. Beats very strong.

1.24.0 Frequency 150 per minute. Beats very strong.

11.25.0 Frequency 150 per minute. Beats very strong.

11.31.0 Frequency 150 per minute. Beats very strong.

11.40.0 Frequency 140 per minute. Beats apparently not stronger than is normal.

11.45.0 Rhythm as above; *.003 of a c.c. of ammonia given.*

11.47.0 Frequency 40 per minute. Heart in an expanded condition.

12.10.0 Heart in an expanded condition giving feeble systolic twitches.
Temperature of the atmosphere of the incubator was here raised to 42° C.

12.11.0 Frequency 100 per minute; but beats very feeble.

12.11.30 *.003 of c.c. of ammonia given.*

12.14.0 Systoles too weak to count. Heart in an expanded condition.

12.15.0 The ventricle gave 100 twitches, but was in an expanded condition.

12.22.0 The ventricle gave about 120 twitches during the minute.
They were extremely feeble; the heart being in an expanded condition.

12.24.0 *.005 c.c. of ammonia solution.*

12.27.0 Ventricle extremely dilated, but gave 100 twitches during the minute.

12.28.0 *.009 c.c. of ammonia solution given.*

12.29.0 The twitches of the dilated ventricle are too feeble to record.

12.30.0 Heart stopped in extreme diastole.

12.31.0 1 c.c. of normal saline containing *.005 c.c. of glacial acetic acid was added.*

12.32.0 No change in heart's condition. 3 c.c. of normal saline containing *.015 c.c. of glacial acetic* was added. No change in the tonic condition of the heart was observed.

As noted in the historical summary Schenk found that the vapour of ammonia stopped the embryonic heart. It is evident from the preceding experiment Schenk's dose was far too large and only gave the final action recorded above without the primary acceleration. It will also be noted that small doses of ammonia do not influence the frequency of the embryonic heart. After further administration a phase ensues when the frequency is diminished, and as far as I am able to judge without a graphic record is associated with an increase of force of the beats. A still further increase of the dose which in the case of the experiment recorded in this paper had reached a total of *.005* of a c.c. of strong ammonia caused the frequency of the heart to increase greatly

—30 beats per minute above the normal—, while the beats were apparently much stronger than in the normal condition. A further increase of .003 c.c. of strong ammonia in 1 c.c. of normal saline introduced the final stage of depression in which the whole heart was expanded. Several attempts were made to restore the heart to its normal condition by the application of varying quantities of acetic acid at different phases of the action of the ammonia, but with negative results. It therefore seems that action of ammonia on the cardiac myoplasm is different from the action of simple alkali which Gaskell¹ has antagonized by acid on the adult heart and which is also antagonized by acid on the embryonic heart. A curious effect of large and fatal doses of ammonia is the rupture of the vessels of the blastoderm. Thus in a 70 hour embryo with a powerful rhythm of 150 beats per minute, 1 c.c. of normal saline containing .006 c.c. of strong ammonia stopped the heart in extreme expansion, at the same time causing local haemorrhages in the blastoderm. Since the expanded condition of the heart shews that its action is not producing a high tension in the capillaries, this result is probably due to direct action on the capillaries themselves.

The stimulant effect of ammonia on the embryonic heart is similar to that recognized by Brunton² on the adult heart.

Ringer³ has shewn with the frog's ventricle that the introduction of ammonia into the circulating fluid produces undulations in the cardiac trace during the diastolic pause, while "under larger and larger doses the diastolic dilatation after each rhythmic contraction becomes less and less, until finally the diastolic dilatation fades away, and the trace becomes a slightly wavy line."

These observations are in marked contrast with the extreme diastolic dilatation produced by the action of ammonia on the embryonic myoplasm. Whether this difference of action is owing to the action of the ammonia on the fine nerve terminations which may exist in the ventricular substance of the adult heart, or whether it is due to an intrinsic difference of the two forms of contractile tissue I am unable to decide. The difference between the two types of action is a difference of tone and will be further considered in section on tone.

The following experiment was made with tetraethyl-ammonium

¹ Gaskell. *This Journal*. Vol. III. p. 57.

² Brunton. *Text-Book of Pharmacology*. (3rd ed.), p. 328.

³ Ringer. *This Journal*. Vol. III. p. 195.

hydroxide in which four ethyl groups replace the four hydrogen atoms of the ammonium base.

Dec. 3rd, 1892. Embryo aged 60 hours. Temperature throughout the experiment 38° C. Normal rhythm 74 per minute.

At 11.15.0 1 c.e. of normal saline containing .00075 c.c. of tetraethyl-ammonium hydroxide was added.

11.16.0 The rhythm remained unchanged.

11.17.0 The rhythm remained unchanged.

11.27.0 The rhythm the past ten minutes remained normal.

11.32.0 The rhythm during the past five minutes remained normal. .0002 c.c. of the substance was given.

11.34.0 No change in the rhythm.

11.38.0 Rhythm unchanged. .012 c.e. of the body given.

11.39.0 Heart passed into an expanded condition, in which it was irresponsible to stimuli.

The above experiment shews that the general action of tetraethyl-ammonium hydroxide on the embryonic heart is similar to that of ammonium hydroxide. Owing to my supply of the former substance failing I have been unable to make further observations to see if a phase of stimulation occurs in its action.

It is however evident that the introduction of the ethyl groups into the ammonium molecule does not influence its action on the tone of the embryonic heart.

On the Action of Veratrine.

H. C. Wood¹ who made numerous experiments on the action of sulphate of veratrine on mammals and birds found that it depressed their cardiac rhythm. This primary depression was often followed by an increase of both force and frequency of the rhythm which were however attributed by Wood to the asphyxia that was setting in. In some of the experiments on pigeons the heart beats become too frequent to record.

Ringer² has found that veratrine when introduced into the frog's ventricle lengthens out its systole and by depressing the reparative processes of the ventricle stopped it in diastole. He also noted that veratrine has a more powerful action in hot than in cold weather. In hot weather it causes irregularities, and in larger doses even

¹ Wood. *Amer. Jour. Med. Sci.* Vol. LIX. (1870), p. 36.

² Ringer. *This Journal.* Vol. v. pp. 46 and 353. *Practitioner.* Vol. xxx. (1883), p. 17.

ineordination of the heart's rhythm. To Ringer we also owe the discovery that veratrine and potassium chloride are reciprocally antagonistic in their action on the frog's ventricle, and that this antagonism is complete in cold weather, while in hot weather though potassium chloride will completely antagonise veratrine yet potassium chloride is only partially antagonised by veratrine and from these observations he concludes that there is probably a limit to antagonism in warm blooded animals.

My results with veratrine on the embryonic heart are illustrated by the following experiments.

Oct. 28, 1892. Embryo aged 72 hours. Temperature throughout the experiment 38°C . Normal rhythm 92 per minute.

At 11.1.25 1 c.c. of normal saline containing '0001 *gram of veratrine* was given.

11.2.25 Frequency was 96 per minute. Rhythm otherwise normal.

11.3.25 Rhythm as above.

11.4.25 Rhythm as above.

11.5.25 Rhythm as above.

11.6.30 A second e.e. containing '0001 *gram of veratrine* given.

11.10.0 Rhythm during the last four minutes unchanged.

11.11.0 Frequency fell to 82 per minute. No other apparent change in the rhythm.

11.15.0 Rhythm during last four minutes constant at 82 per minute.

11.15.5 A third dose of '0001 *gram of veratrine* was given.

11.16.5 Frequency 100 per minute. Rhythm apparently otherwise unchanged.

11.17.0 Frequency 104 per minute. No other change in rhythm.

11.18.0 Frequency 102 per minute.

11.19.0 Frequency 76 per minute. The systoles are slightly drawn out.

11.22.0 During the last five minutes the rhythm remained as in the preceding observation.

11.23.0 1 c.c. of normal saline containing '0002 *gram of veratrine* was given.

11.25.0 Frequency 52 per minute. The systoles are very drawn out.

11.30.0 During the last five minutes the rhythm exhibited some irregularities varying in frequency from 58 to 52 beats per minute. The systoles were very drawn out.

11.30.10 A dose of '002 *gram of veratrine* in 1 c.c. of normal saline given.

11.31.0 Frequency 82 per minute. Systoles drawn out.

11.32.0 Frequency 70 per minute. Systoles drawn out.
 11.33.0 Frequency 62 per minute. Systoles more drawn out.
 11.35.0 Frequency 58 per minute. Same remark as above.

At this juncture the temperature of the air of the incubator was rapidly raised to 46° C. The heart resumed its normal rhythm and the effect of the veratrine being removed by heating.

The action of veratrine on the embryonic heart may be summarized by stating that doses of .0001 gram increase the number of beats per minute. Larger doses may cause temporarily an increase of rhythm, but soon depress the heart by gradually lengthening the systole which becomes very weak, while the diastole is complete. The whole heart stops in an expanded condition.

We note that this result agrees closely with that of Ringer on the frog's ventricle. With regard to the phase where the frequency of the rhythm is increased it does not correspond to great increase observed by Wood on adult hearts whose explanation is probably not incorrect.

The fact that heat destroys the action of veratrine on the embryonic heart, corresponds to the same observation made by Ringer (*loc. cit.*) on adult cardiac muscle and the observations made by Brunton and Cash¹ on skeletal muscle.

It is also interesting to note that Romanes² has recorded a primary phase of excitation in the action of veratrine upon medusæ, during which both the force and frequency of the rhythm is increased. This phase gives place to one in which the beats gradually diminish till the animal rests in diastole. It is therefore probable that veratrine acts directly upon the contractile tissue of the medusæ and not viâ the nervous rete. According to Plateau³ veratrine causes a primary acceleration and increased potency followed by depression and diastolic death on the heart of *Carcinus mœnas*. A similar augmentation of the beats was described by the same observer on the heart of *Homarus*, but the stoppage was sometimes in systole, at others in diastole. An acceleration followed by typical depression and diastolic death was recorded by Yung⁴ on the hearts of lamellibranchs, and Krukenberg⁵

¹ Brunton and Cash. *This Journal.* Vol. iv. pt. 3. *Centralbl. f. d. med. Wiss.* 1883, No. 6.

² Romanes. *Phil. Trans. Roy. Soc.* Vol. 167, p. 736.

³ Plateau. *Bulletin de l'Acad. Roy. des Sciences &c. de Belgique.* 2^{me} série, T. XLVII. (1878), p. 207.

⁴ Yung. *Archiv Expér. de Zool.* T. ix. (1881), pp. 429—444.

⁵ Krukenberg. *Vergleichend-physiologische Studien.* Heidelberg, 1880. S. 168.

on that of the crayfish. While the general action of veratrine seems constant throughout the vertebrates examined, there seem to be some variations within the group crustacea. This fact emphasises the point urged in the opening section of this paper that it is dangerous to draw inferences from observations on a single group of animals and that the comparative method is the most reliable.

My results on the antagonism of veratrine and potassium chloride on the embryonic heart are illustrated by the following experiment.

Nov. 7th, 1892. Embryo aged 72 hours. Temperature during the experiment 40° C. Normal rhythm 128 per minute.

At 12.29.10 1 c.c. of normal saline containing '0005 gram of veratrine was given.

- 12.30.0 Frequency 120 per minute.
- 12.32.0 Frequency 100 per minute. Beats irregular but strong.
- 12.34.0 Frequency 68 per minute. Beats irregular but strong.
- 12.35.0 *Heart stopped in diastole.*
- 12.36.0 1 c.c. of normal saline containing '01 gram of potassium chloride was given.
- 12.37.0 Heart recommenced beating.
- 12.38.0 Frequency 80 per minute. Beats weak.
- 12.39.0 Frequency 96 per minute. Beats stronger.
- 12.42.0 Frequency 72 per minute. Beats apparently normal. This rhythm was maintained for a considerable period of time, until the observation terminated.

I therefore conclude that the antagonism between veratrine and potassium chloride is due to the direct action of the substances on the myoplasm of the embryonic heart and on the cardiac fibres of the adult heart. It also affords additional evidence of the similarity between the contractile substance of the heart of the embryo and adult in their response to chemical stimulation¹.

I have failed to obtain the converse antagonism between potassium chloride and veratrine.

The higher temperature which is normal in the chick embryo, seems to correspond to frog's heart when warmer, due to a higher temperature of the surrounding atmosphere, under which conditions its metabolism seems to approach nearer to the cardiac metabolism of the

¹ If the embryonic heart is stopped by larger doses of veratrine as for instance by '003 gram, then potassium chloride fails to restore the rhythm.

chick embryo. Another factor in this result may be the greater activity of the veratrine acting at the somewhat higher temperature.

Therefore there seems to be a temperature which in both the frog and chick is most favourable for the greatest action of veratrine on their respective hearts, while the temperature above and below this is less favourable. The most favourable temperature is not the same for both types.

Action of Chloroform and Ether.

Claude Bernard¹ expressed his opinion thus on the relative action of chloroform and ether on the heart. "As to ether and chloroform, their action is almost the same from a physiological point of view, excepting there is a difference of intensity in the favour of chloroform." Since this period and up to the present time there has been a wordy discussion on the action of these bodies and it is only within the scope of this paper to quote the two most divergent schools of thought.

McWilliam² writes, "There is commonly seen a very striking and important difference between the relative influence of the two anæsthetics upon certain functions. Ether can abolish the conjunctival reflex and induce profound anæsthesia with no appreciable direct influence on the heart; while chloroform in causing a less deep anæsthesia—in which the conjunctival reflex is not abolished—may cause a marked dilatation of the whole heart." He further states that the weakening and dilating effects of chloroform are sometimes manifested in tolerably equal degree, in both auricles and ventricles, but sometimes, more readily upon the auricles, at other times upon the ventricles.

Lawrie³ denies the importance of McWilliam's observations and believes that any cardiac dilatation obtained was probably due to pulmonary obstruction. McWilliam⁴ replied that in many cases the whole of the heart was dilated and that dilatation of the right side could not be accounted for by pulmonary obstruction⁵.

¹ C. Bernard. *Leçons sur les Anesthésiques et sur l'Asphyxia*, p. 101. Paris, 1875.

² McWilliam. *Scientific Grants Committee of British Med. Assoc. Brit. Med. Jour.* Oct. 11th, 18th and 25th and Nov. 1st, 1890.

³ Lawrie. *Medical Chronicle*, Jan. 1891.

⁴ McWilliam. *Medical Chronicle*, Feb. 1891.

⁵ A full account of the discussion is reprinted in the *Report of the Hyderabad Chloroform Commission*. Bombay, 1891. See also Gaskell and Shore, *British Med. Jour.* 1893. Jan. and Feb. paper in progress while this was being written.

He has also recently published his tracings¹, and answered certain objections that have been raised against his views on the cardiac dilatation said to be induced by chloroform. He writes, "It is not obviated by the section of the vagus nerves, nor does it depend upon a fall of systemic arterial pressure. For evidences of slight expansion begin to shew themselves before the pressure begins to fall. And the fact that a fall of pressure brought about by means not directly affecting the heart (e.g. section of vasomotor nerves) causes no dilatation."

The following are my results with dilute application of chloroform and ether dissolved in normal saline to the embryonic heart.

A. 16th Dec., 1892. Embryo 75 hours. Temperature throughout the Experiment 35° C. The normal rhythm was 120 per minute.

At 11.0.0 $\frac{3}{4}$ of a c.c. of chloroform solution was added.

- 11.2.0 Frequency reduced to 110 per minute. Diastole exaggerated.
- 11.3.0 Frequency reduced to 110 per minute. Diastole exaggerated.
- 11.5.0 Frequency reduced to 110 per minute. Diastole exaggerated.
- 11.7.0 Frequency rose to 114 beats apparently normal.
- 11.9.0 When frequency was still 114 1 c.c. of the chloroform solution was added.
- 11.10.0 Frequency still 114 but heart in an expanded condition.
- 11.16.0 Frequency 114 and heart resumed normal condition.
- 11.18.0 $1\frac{1}{2}$ c.c. of the chloroform solution applied.
- 11.19.0 Frequency 108. Heart in an expanded condition, showing irregularities of rhythm.
- 11.21.0 Frequency of ventricle 90, but auricle beating at a less frequent rhythm. The whole heart in a dilated condition.
- 11.23.0 Frequency recovered to 114 and beats normal.
- 11.24.0 2 c.c. of chloroform solution added.
- 11.25.0 Heart in an expanded condition with about 80 twitches of the ventricle in the minute. The auricle twitches still more feebly.
- 11.26.0 Irregular rhythm, ventricle beating more than auricle.
- 11.26.30 Rhythm reversed.
- 11.27.0 Fluttering reversed rhythm of about 140 per minute.
- 11.28.0 Fluttering reversed rhythm of about 140 per minute.
- 11.29.30 Auricles and ventricles apparently beating simultaneously.
- 11.30.0 Rhythm reversed back to the normal sequence in auricle and ventricle.

¹ Mc William. This *Journal*. Vol. xiii. 1892, p. 860.

11.31.0 Very feeble rhythm of 102, normal in direction, heart in an expanded state.

11.33.0 Very feeble rhythm of 110 normal in direction, heart in an expanded state.

11.34.0 Auricle stopped in extreme dilatation, ventricle gave 110 very feeble twitches and stopped in diastole.

11.35.0 Auricle gave few feeble twitches and rhythm reversed.

11.37.0 Reversed rhythm of 130 and the beats fairly good and the heart but slightly dilated.

11.42.0 Reversed rhythm in ventricle of 140 but feeble. The auricle is very dilated and beating much less frequently.

11.45.0 Reversed ventricular rhythm of 120, which passes more often into the auricles.

11.47.0 Heart reversed back to normal rhythm of 120. Auricle very dilated, ventricle less dilated.

11.49.0 Rhythm of 120 normal in direction but very weak. Heart extremely expanded.

11.51.0 2 c.c. of chloroform solution added.

11.52.0 Auricle stopped in extreme dilatation, ventricle twitches but is dilated.

11.53.0 Same condition of auricle, a few feeble twitches of the ventricle.

12.10.0 Heart stopped in extreme dilatation of both auricle and ventricle.

It was pointed out by Ringer¹ that if a frog's heart be stopped in diastole by chloroform the application of a one per cent solution of ammonium hydrate will restore the beats almost to their original power. In the following experiment I have tried this antagonism on the embryonic heart.

Dec. 16th, 1892. Embryo aged 80 hours. Temperature throughout the experiment 30°. Normal rhythm 140 per minute.

12.50.0 3 c.c. of chloroform solution administered.

12.53.0 Frequency 135 per minute. Rhythm normal.

12.55.0 Frequency 135 per minute. The systoles are fluttering and incomplete. The heart is in an expanded condition.

12.56.0 4 c.c. of chloroform solution administered.

12.57.0 Heart in extreme expansion. Only a few twitches of its substance.

12.58.0 Auricle dilated, ventricle beating fairly well.

1.0.0 Heart still expanded but beating with a frequency of 135 per minute.

¹ Ringer. *Practitioner*. Vol. xxvi. (1881), p. 436.

1.1.0 4 c.c. of chloroform solution added.

1.3.0 Heart in extremely dilated condition, auricle alone twitching.

1.5.0 Heart still very dilated, auricles stopped, ventricle twitching feebly.

1.7.0 1 c.c. of solution of ammonia administered.

1.8.0 Ventricular twitches pass to auricle.

1.9.0 The beats of both auricle and ventricle become more powerful.

1.10.0 Ventricular beats become more powerful.

1.12.0 The rhythm is still reversed but has improved in force.

1.14.0 The auricle beating now before the ventricle. Rhythm of the latter 80 per minute with good systoles and diastoles.

1.16.0 2 c.c. of chloroform solution administered.

1.17.0 Heart again passed into dilated condition, auricle stopped, ventricle beating.

1.19.0 Auricle beating very feebly, ventricle beating well.

1.21.0 Auricle stopped in dilatation, ventricle beating fairly well but dilated.

1.23.0 1 c.c. of chloroform solution administered.

1.27.0 Whole heart in extreme dilatation, with an occasional ventricular twitch.

1.28.0 2 c.c. of ammonia solution administered.

1.30.0 Heart still in extreme dilatation.

1.30.10 Ventricle commences to beat with slow and complete systoles, which pass partially to the auricle.

1.31.10 Occasional strong ventricular systoles which do not spread to the auricle.

1.35.0 Occasional ventricular twitches which do not spread to the expanded auricles. Ventricle also greatly expanded.

1.40.0 Same condition of the heart.

My results with ether are illustrated by the following experiment.

6th Dec., 1892. Embryo aged 75 hours was exposed to temperature of the laboratory in a moist chamber. The temperature of 14° stopped the heart in diastole. The embryo was restored to the observing apparatus which was at a temperature of 32°. The heart after 10 minutes in the apparatus had not resumed beating. The experiment then was commenced.

11.45.10 1 c.c. of normal saline containing .0033 c.c. of ether was administered.

11.46.0 Heart starts beating.

11.47.0 Heart has a regular rhythm of 120 per minute.

11.49.0 Heart has a regular rhythm of 120 per minute.

11.53.0 Heart has a regular rhythm of 130 per minute.

11.57.0 Heart has a regular rhythm of 130 per minute.

11.58.0 2 c.e. of solution containing .006 c.e. of ether was administered.

11.59.0 Regular heart rhythm of 180 per minute.

12.15.0 Regular heart rhythm of 132 per minute.

12.17.0 Regular heart rhythm of 132 per minute.

12.18.0 1 c.e. of solution containing .0033 c.e. of ether was administered.

12.19.0 12.20.0, 1.21.0, 12.25.0. Each had a rhythm of 170 per minute.

12.30.0 Same rhythm, 2 c.e. of solution administered containing .006 c.e. of ether.

12.32.0 Heart rhythm 170 per minute.

12.41.0 Heart rhythm 130 per minute.

12.48.0 Heart rhythm 130 per minute.

12.53.0 1 c.c. of solution containing .045 c.e. of ether administered.

12.54.0 Frequency 140 per minute. Heart expanded and beats feeble.

12.57.0 1 c.e. of solution containing .06 of c.e. of ether administered.

12.58.0 Heart stopped in diastole and gave two beats during the minute.

12.59.0 Does not respond to mechanical stimuli.

1.10.0 Heart evidently dead in diastole.

From the above experiments it is evident that there is a marked difference in the action of ether and chloroform on the embryonic heart. Chloroform depresses the rhythm to diastolic stoppages of both auricle and ventricle, though it seems to be more toxic to the auricular substance than to the ventricular substance. Ether in moderately large doses stimulates the embryonic heart and only after very large doses kills it in diastole.

In my experiments there are obviously no complications possible owing either to increased arterial tension or to the influence of a nervous mechanism. The objection that might be raised to applying results from the embryonic to the adult heart, will I trust be removed by considering the remarkable similarity of the action of poisons of the digitalin group on the embryonic myoplasm, to their action on the adult cardiac fibre. It may also be remarked that adult cardiac fibres are in many respects primitive; as for instance, their transverse striations are but feebly marked. These observations therefore favour the views advanced by McWilliam on the essential differences of the action of chloroform and ether on cardiac fibres, but of course do not give any answer, as to at what stage in chloroform anaesthesia

dilatation is liable to set in. The partial recovery that takes place in ventricular myoplasm that has been previously poisoned by chloroform, on the addition of ammonia, is in accord with the experiments cited above by Ringer on the frog's ventricle.

We may here record the difference between the embryonic heart and that of decapod crustaceans under the influence of chloroform. The ultimate depression of the frequency and force of the cardiac rhythm of the latter is preceded by a stage where the beats become more ample¹. This period of exaltation of the rhythm is absent in the embryonic heart.

Muscarin and Atropin.

The intimate relationship between the action of these two drugs renders their consideration together desirable.

A. *Muscarin*. The earlier phenomena observed with this substance on the heart and its antagonism with atropin were ascribed by Schmiedeberg⁽¹⁾ and his school to its action on the "motor ganglia" of the heart. Owing chiefly to the researches of Gaskell⁽²⁾ who shewed that muscarin acted like an acid solution, and atropin like an alkaline solution, many physiologists now regard their action as direct upon the cardiac muscle. The present view of the subject is thus summarized by Foster,⁽³⁾ "we have evidence that atropin acts either on the muscular tissue itself or on the very endings of the nerves in the muscular fibres. And as in the case of atropin, so in the case of muscarin, there is evidence that the drug acts not on any ganglionic mechanisms but on the cardiac tissue itself."

Löwit⁽⁴⁾ who has made experiments on the conjoint action of strychnine and muscarin, and who also states that strong doses of curare do away with the muscarin standstill concludes that "das Muscarin in erster Linie die nervösen Apparate des Herzens überhaupt, und erst in zweiter Linie den Herzmuskel beeinflusst."

Ransom⁽⁵⁾ who worked with the hearts of cephalopods found that one milligram of muscarin stopped their hearts in diastole, the stoppage being often preceded by the appearance of Luciani's beats. The stoppage occurs even after the animal has been curarized but a "further addition of moderate or a rather large dose of curare may frequently restore the rhythm overthrown by muscarin." Ransom believes that the antagonism is due to both poisons acting on the muscle.

Fano⁽⁶⁾ states that mechanical excitation of the auricles and more

¹ See F. Plateau. *Association Française des Sciences.* 7^e session, 1878, p. 735.

rarely of the ventricles of the frog causes variations of the cardiac tone. Muscarin paralyses the systolic function of the heart, but leaves intact the tonic function. On the contrary these oscillations of cardiac tone are destroyed by atropin, nicotine and veratrine.

Yung⁽⁷⁾ experimenting with the heart of *Mya arenaria* found that after a muscarin injection the cardiac rhythm rose from the normal six beats per minute to twelve beats per minute. Krukenberg⁽⁸⁾ who used muscarin nitrate of Merck's manufacture and who performed control experiments on the hearts of frogs found that local injection or direct application to Salpas' hearts (Ascidians) did not influence their rhythm. Roy and Adami⁽⁹⁾ write of the action of muscarin on the mammalian heart "that complete arrest of the auricles, the ventricles being left entirely to their own intrinsic mechanism does not itself necessarily cause temporary arrest of the ventricles such as may be produced by strong vagus action."

In a preliminary communication to the Royal Society I pointed out that muscarin nitrate has no specific action on the hearts of chick embryos. Since writing that paper I have found that two observers whose observations seem to have been neglected in England have obtained similar results, Krukenberg in a footnote of his work writes "Sowohl Atropin als Muscarin sind, wie ich an einem andern Orte ausführlicher mittheilen werde, auf das lebhaft pulsirende Herz des Hühner-Embryos in seinen ersten Entwickelungsstadien unwirksam," while Kober⁽¹⁰⁾ states that muscarin has no action on the heart of chick embryos in all stages of their embryonic life, but that on the heart of chicklings just hatched it shews its typical action.

Kober has also made observations on tadpoles' hearts and finds that they are typically affected by muscarin. He concludes that in the free-swimming condition, the nervous mechanism of the heart is more developed, and that in the protected life of incubating egg it remains in a retarded condition. My results with muscarin nitrate which was obtained from Martindale's and which was shewn to be active by the performance of control experiments with frogs' hearts at the same time as the embryo-experiments were made are illustrated by the following tables.

I. Nov. 18th, 1892. Embryo aged 75 hours. Temperature throughout the experiment 30° C.¹. Normal frequency 40 beats per minute.

¹ We may here remark that temperature is subnormal in the chick and therefore the absence of muscarin effects is not the result of a high temperature, since Petri⁽¹¹⁾ has shewn that heating a frog's heart removes the muscarin standstill.

At 12.30.0 1 c.c. of normal saline containing $\frac{1}{4}$ c.c. of *saturated solution of muscarin nitrate*.

12.32.0 Frequency 41 per minute, no observable change in the rhythm.

12.34.0 Frequency 41 per minute, no observable change in the rhythm.

12.35.0 Frequency 41 per minute, no change.

12.36.0 2 c.c. of saline containing $\frac{1}{2}$ c.c. of *saturated muscarin nitrate*.

12.37.0 Frequency 41 per minute. No change in the rhythm.

12.45.0 Rhythm during last eight minutes unchanged.

12.50.0 Rhythm during last five minutes unchanged.

II. Nov. 24, 1892. Embryo aged 72 hours. Temperature throughout the experiment 35° C. Normal frequency 92 per minute.

At 12.6.0 2 *drops* of half saturated solution of *muscarin nitrate* added.

12.7.0 Frequency 92 per minute and rhythm unchanged.

12.8.0 Frequency 92 per minute and rhythm unchanged.

12.9.0 Rhythm unchanged. 2 *drops* of half-saturated solution of *muscarin nitrate* added.

12.13.0 Rhythm during last four minutes was unchanged at 92 beats per minute. 4 *drops* of half-saturated solution of *muscarin nitrate* were given.

12.16.0 Rhythm during last three minutes unchanged.

12.17.0 2 *drops* of half-saturated solution of *muscarin nitrate* were given.

12.20.0 Rhythm unchanged. 2 *drops of saturated solution of muscarin nitrate* added to the fluid over the embryonic heart. It stains the embryo brown.

12.21.0 Frequency 91 per minute. Rhythm unchanged.

12.22.0 Frequency 86 per minute. Rhythm unchanged.

12.23.0 3 *drops of saturated solution of muscarin nitrate* given.

12.27.0 Rhythm during last four minutes was 82 per minute. Beats normal. 2 *more drops* given.

12.28.0 Rhythm unchanged at 82 per minute.

12.30.0 Beats slightly irregular. Frequency 68 per minute.

12.32.0 Beats slightly irregular. Frequency 80 per minute.

12.34.0 Beats regular and frequency 78 per minute.

12.37.0 2 *drops* of saturated solution added.

12.40.0 Frequency 65 per minute and rhythm shewing no abnormalities.

III. Nov. 25th, 1892. Embryo 100 hours. Temperature 40° C. Normal frequency 96 per minute.

11.17.0 1 *drop* of saturated solution of *muscarin nitrate* in 1 c.c. of normal saline given.

11.20.0 Frequency 96 per minute. No change in characteristics of the rhythm.

11.22.0 Frequency 96 per minute. No change in characteristics of the rhythm.

11.23.0 The rhythm became irregular and reversed, frequency was 76 beats per minute, but there were in the middle of the observation diastolic pauses of about two seconds each.

11.26.0 Rhythm during last two minutes was normal at 96 beats per minute.

12.26.0 Rhythm normal. 3 drops of saturated solution added.

12.29.0 Frequency during last three minutes 100 beats per minute. No change in the rhythm.

12.33.0 No change from above record.

12.34.0 2 drops of saturated solution given.

12.40.0 No change from above record.

The direct application of saturated solution of muscarin nitrate to the embryonic heart stops its beating, at the same time coagulating the surrounding tissues. Probably any strong alkaloidal substance would produce the same result. The abolished rhythm is not restored by the application of atropin, neither are there any typical muscarin phenomena.

B. *Atropin.* Von Bezold and Bleobaum⁽¹²⁾ found that with small doses of atropin there was an acceleration of the heart, and they concluded that atropin both paralyses the endings of the vagi and also acts on the cardiac muscle.

Of frogs Meuriot⁽¹³⁾ wrote, "Chez ces animaux l'atropine paraît agir directement sur le muscle cardiaque dont il détruit l'excitabilité."

Gaskell¹ shewed that if atropin were applied to sinus and auricles it depressed both auricles and ventricles, but that if applied to the ventricle alone it had only a slight effect. To him also we owe the discovery that when a heart is stopped by atropin there is a positive electromotive variation of the cardiac, comparable to the positive electromotive variation concurrent with the standstill resulting from stimulation of the vagus while a negative electromotive variation accompanies the muscarin standstill. Gaskell leans to the view that the action of atropin and consequently of muscarin is direct upon the cardiac muscle.

Romanes⁽¹⁴⁾ who experimented upon Sarsa and Tiaropsis amongst the medusæ, found that in Sarsa atropin caused the swimming movements to become convulsive. The systoles next became feeble, and

¹ An abstract of Gaskell's work is omitted, since all physiologists are or should be conversant with it in the original. Only those points touched in the critical portion of this paper are mentioned.

finally ceased. The nectocalyx and manubrium passed into tonic contraction, but muscular irritability remained after tentacular irritability had ceased. In *Tiaropsis* the characteristic action was convulsive bouts of irregular systoles. Schotten⁽¹⁵⁾ stated that atropin diminished the heart-beats of the lobster. Jordan⁽¹⁶⁾ stated that atropin did not influence the lobster's heart. Schotten's statement has recently been confirmed by Plateau⁽¹⁷⁾ who with small doses of atropin obtained a primary acceleration, with larger doses a considerable depression of the heart's action. With a dose of .05 gram he depressed a normal rhythm of 120 beats per minute to 74 beats per minute. Dolgied⁽¹⁸⁾ found that a solution of atropin sulphate (strength not given) caused only a slight diminution in the frequency of the cardiac rhythm of the larva of *Corethra plumicornis*. Yung writing of the action of atropin on lamellibranch molluses' hearts states, "Le sulphate d'atropin administré à très fortes doses, ne produit pas des effets sensibles sur les Lamellibranchs."

Krukenberg states that neither atropin nor muscarin influence the hearts of *Salpæ* (Ascidians), while helleborin shewed a marked action.

Ransom states that atropin in doses of .25 milligram primarily has a marked stimulant action on the hearts of Cephalopods, it afterwards causes idiomuscular contraction of the cardiac muscle and finally the heart stops in systole. Its action closely resembles that of digitalin on the frog's heart. When atropin is given to a cephalopod heart that has been muscarized it usually only changes the tone of the heart and only in "one or two cases atropin caused a restoration of rhythm."

My results with atropin on the embryonic heart are as follows:

I. Nov. 1st, 1892. Embryo aged 75 hours. Temperature throughout the experiment 35° C. Normal rhythm 112 per minute.

2.59.30	1 c.c. of normal saline containing .012 gram of atropin given.
3.0.0	Frequency 112 per minute. No observed change in the rhythm.
3.1.0	Frequency 104 per minute. No observed change in the rhythm.
3.2.0	Frequency 96 per minute. No observed change in the rhythm.
3.5.0	Frequency 80 per minute. No observed change in the rhythm.
3.5.30	$\frac{1}{2}$ c.c. of normal saline containing .012 gram of atropin given.
3.6.30	Frequency 64 per minute. No observed change in the rhythm.

3.10.30 During last four minutes rhythm remained unchanged. 1 c.c. containing .024 gram of atropin given.

3.13.30 No change in rhythm. $\frac{1}{2}$ c.c. containing .06 gram of atropin given.

3.18.0 The rhythm during the last five minutes shewed no visible change in characteristics and had a frequency of 60 per minute.

3.18.30 $\frac{1}{2}$ c.c. containing .06 gram of atropin given.

3.25.0 During the last seven minutes the rhythm was maintained at 60 per minute.

3.25.30 $\frac{1}{2}$ c.c. containing .06 gram of atropin given.

3.28.0 Rhythm during last three minutes unchanged.

3.28.30 $\frac{1}{2}$ c.c. containing .06 gram of atropin given.

3.35.0 Rhythm during the last seven minutes unchanged.

During this experiment more than .275 gram of atropin was given direct on the embryonic heart and the rhythm was maintained at 64 beats per minute.

II. Nov. 2nd, 1892. Embryo aged 78 hours. Temperature during the experiment 38° C. Normal rhythm 138 per minute.

At 1.14.0 $\frac{1}{2}$ c.c. of saline containing .001 gram of atropin was given.

1.15.0 Frequency 128 per minute. Rhythm apparently otherwise unchanged.

1.17.0 Frequency fell to 110 per minute. No change in characteristics of rhythm observed.

1.18.0 $\frac{1}{2}$ c.c. containing .002 gram of atropin given.

1.22.0 Frequency during last four minutes 90 per minute. No other change in the rhythm observed.

1.23.0 $\frac{1}{2}$ c.c. containing .002 gram of atropin given.

1.26.0 Rhythm the last three minutes unchanged.

1.27.0 $\frac{1}{2}$ c.c. containing .002 gram of atropin given.

1.33.0 Frequency 86 per minute. No change in observed characteristics of the rhythm.

In the above experiment .007 gram of atropin was given direct on to the embryonic heart, and the rhythm was depressed from the normal 138 beats per minute to 86 beats per minute. It will be noted that the primary depression, caused by a comparatively small dose of atropin is relatively much greater than the further depression which results from the addition of much larger doses. These results are in marked contrast with the observations of von Bezold who found in the rabbit that one decigram

of atropin caused not only a cessation of the heart-beats, but a loss of irritability of that organ ; also with those of Ringer and Morshead⁽¹⁹⁾ on the frog, and Gaskell on the frog and tortoise, who found that atropin stopped the rhythmic action of the heart.

C. *Theoretical Conclusions.* It is evident from the preceding observations that to fully understand the action of atropin and muscarin on contractile tissues and their correlated nerve supply (if present) it will be necessary owing to the wide divergences of action on the hearts of different animals to examine its action on a far larger number of types than has at present been done. It also seems that conclusions drawn from the action of these drugs on the hearts of a single class of animals are not necessarily applicable to other classes of animals. This is in marked contrast to the action of drugs of the digitalin group, where there is almost an identical action throughout both vertebrate and invertebrate types. In the case of this group the close resemblance of their action on the embryonic myoplasm, to their action on the adult heart, renders it extremely probable that in the latter case their action is direct on the cardiac fibres. A similar correspondence between the action on the embryonic and adult heart in the caffeine group, in ammonia, veratrine and barium chloride strongly favours the view that the embryonic myoplasm is in its metabolic activity very similar to adult cardiac muscle. Yet in the action of atropin and muscarin there is a marked divergence of action. The statement of Kobert that freshly-hatched chicklings are influenced typically by muscarin shews that the adult avian is influenced by this drug, and excludes the view that there is an intrinsic difference between the metabolic activity of the fowl's heart and those of batrachians, chelonians, and mammals.

If Fano's statements that muscarin leaves intact the tonic variations of cardiac muscle, while veratrine, a drug which acts principally if not entirely upon cardiac muscle, abolishes these tonic variations are correct, it shews that the action of muscarin is more complicated than had previously been considered. Atropin by abolishing tonic variations seems to act more as a muscle poison, and it will be noted that atropin though it acts on the embryonic heart in far less marked a manner than on the adult heart yet it exerts a not inconsiderable action. It is interesting to note that both atropin and curare which paralyse the vagus mechanism (I purposely avoid reference to nerve endings) of the heart have been shewn by Löwit and Ransom (*op. cit.*) to restore the rhythm of inmuscarinized hearts.

Claude Bernard⁽²⁰⁾ has stated that curare paralyses the nerve endings of the hearts of frogs, lizards, and rabbits while it leaves the muscular mechanism intact. I have however found with experiments on frogs' ¹ hearts that if a very large dose of curare be injected into the dorsal lymph sack, it stops the heart in diastole presumably by depressing the rhythmic power of the muscle.

That atropin should act typically on the heart of the lobster is not remarkable since Berger⁽²¹⁾ has found ganglion cells in the posterior portion of its heart. It therefore shews in the possession of both ganglionic and muscular elements a general similarity to the frog's heart. The low differentiation of the nervous system in ascidians is interesting in relation to the statements that neither atropin nor muscarin affect their hearts. It is I think probable from the action of muscarin and atropin on the animals mentioned that the original view of the action of atropin and muscarin via the cardiac ganglia is insufficient to account for the phenomena. As regards the rival view which reduces the ganglia to comparative insignificance in the rôle of the heart's action and exalts the importance of the cardiac fibre, from the facts before me, especially the evidence of the embryonic heart, I am inclined to think that besides the very important factor of the inherent rhythmic function of the cardiac fibres, there may be variations in the action of the heart produced by direct chemical stimulation of the terminations of the cardiac nerves. It is not improbable that muscarin and atropin act primarily on these terminations, and that the paralysis obtained so markedly by the action of atropin and muscarin on batrachian and chelonian hearts is secondary. This is supported by the statement cited from Gaskell that the ventricular tissue is affected much less by atropin than is the auricular tissue, and presumably the nerves are more scantily distributed in the ventricular substance than in the auricular substance. I will also call attention to observations quoted from Roy and Adami on muscarin. Also in connection with this hypothesis I may remark the resemblance of the action of atropin to a vagus standstill and of the action of muscarin to a sympathetic standstill, both of which are probably produced via the termination of those nerves on the cardiac muscle, and in the case of the sympathetic there are valid reasons to believe without the intervention of ganglion cells within the substance of the heart. This shews that the nerve terminations when thrown into natural activity by a "normal nerve stimulus" produce effects similar to those caused by the action of atropin and muscarin; as

¹ The central nervous system of animals experimented on was destroyed.

it is well known that a weak interrupted electric current if applied to a strip of auricular tissue that is rhythmically contracting, will inhibit the rhythm, while if applied after bathing the strip with a dilute solution of atropin the inhibition cannot be produced. As Foster has pointed out the weak interrupted current may have stimulated the fine nerve terminations in the muscle, and "the atropin produced some effect either on these fine fibres or on their connections with the muscular substance, or on the actual muscular substance itself by virtue of which they ceased to act."

It is therefore possible reasoning from the data afforded by the frog's heart, exclusive of the evidence of the embryonic heart, that atropin and muscarin act *via* the fine nerve terminations and not direct on the cardiac muscle.

I am commencing experiments on the action of constant and interrupted currents on the embryonic hearts of chicks and mammals and I reserve a definite conclusion on this point for the future. I also hope to try the action of atropin and muscarin on various forms of rhythmically contractile protoplasm (*e.g.* volvox, other algæ, cilia etc.) and hope by the comparative method to throw some light on this vexed question.

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Strychnine.

The action of this drug on the embryonic heart is shewn by the following summary which is taken from my previous paper.

Strychnine was given to a seventy hours' embryo in a dose of .000017 gram, and depressed the rhythm of the heart from 112 per minute. There was no spasm. In an eighty hours' embryo at 39° C. a dose .00002 gram temporarily increased the rhythm both in force and frequency; then the systole rapidly became weakened and the rhythm irregular. A further dose of .00002 gram still more rapidly reduced both force and frequency of beating, till death in diastole occurred. There was not at any phase of any of the experiments either a spasm or a tetanoid condition.

Romanes (*op. cit.*) who experimented on medusæ with strychnine found that they were thrown into violent spasms, but that death after subsidence of the spasms was in an expanded condition. Presumably the primary phase of spasm was due to the action of the drug on the nervous rete in the medusa's tissues. According to Milner-Fothergill¹ strychnine has no action on the frog's heart.

Brunton and Cash² state that strychnine stimulates the motor ganglia of the frog's heart, since they found that when a frog is under the action of strychnine a ligature placed between the sinus venosus

¹ Milner-Fothergill. *Hasting's Prize Essay*, 1871.

² Brunton and Cash. *St Bartholomew's Hospitl. Rep.* Vol. xvi.

and auricle did not stop the auricle and ventricle as in Stannius' experiment, and if this experiment had already been performed, strychnine injected into the ventricle causes the auricle and ventricle to recommence beating. Löwit¹ has also recently shewn that strychnine when administered to frogs in certain doses entirely annuls the action of the vagus upon the heart. Amongst invertebrates, Yung² experimenting on Mya, a lamellibranch mollusc, found that strychnine injected into the animal's body usually caused a diminution in the frequency and force of its heart-beats, but that the result was inconstant. Direct application to the heart reduced the number of the pulsations, culminating in diastolic stoppage in fifteen to thirty minutes. There was no cardiac spasm described. Plateau³ describes a similar depression and diastolic stoppage in the lobster's heart, while Dolgiel⁴ states that strychnine does not act on the heart of the larva of *Corethra plumicornis*.

The comparison of this series of results shews that there is a marked divergence between the action of strychnine on the heart and its action on the contractile tissue of the medusæ. This seems to shew that its action on the ganglia of the adult frog's heart (if any) is different from its action on the ganglionic cells of the medusæ. Indeed the action of strychnine on the heart resembles its general depressant action on protoplasm (e.g. leucocytes⁵). In considering the experiments of Brunton and Cash and of Löwit in the light of the experiments on the embryonic heart it seems necessary in explaining the former results to bear in mind that strychnine has probably a direct action upon the adult cardiac fibre as well as any action it may have on the ganglia. Possibly the prevention of Stannius' experiment cited by Brunton was due to a primary excitant action direct on the cardiac fibre since strychnine shews a primary excitant action on the embryonic myoplasm and also in Löwit's experiments it is possible that the failure of vagus action may in part be due to direct depressant action of the larger doses of strychnine on the cardiac muscle.

The rhythm of an embryonic heart that has been depressed by strychnine was restored above its normal frequency by raising the temperature 10° C. Further doses of strychnine are required in order

¹ Löwit. *Pflüger's Archiv.* Bd. xxviii. (1882), S. 312.

² Yung. *Archives Expér. de Zool.* T. ix. (1881), pp. 429—444.

³ Plateau. *Archives de Biologie* (Van Beneden's). T. i. (1880), p. 667.

⁴ Dolgiel. *Mém. de St Pétersbourg Acad.* vir^e série. T. lxxiv. No. 10, 1877, p. 16.

⁵ Brunton. *Pharmacology*, p. 61.

to again depress the rhythm. Thus strychnine acts more powerfully on the embryonic heart at lower temperatures than at a higher temperature. It will however stop the heart even after the temperature has been raised to 45° C. the stoppage being always in diastole.

Since the stoppage is always atonic, I tried the effect of caffeine, which stops the heart in a contracted condition. I have however failed to antagonise the action of strychnine on the embryonic heart by doses of caffeine.

Pilocarpine.

My results with pilocarpine are illustrated by the following experiments.

Nov. 21st, 1892. Embryo aged 60 hours. Temperature during the experiment 35° C. Normal rhythm 82 per minute.

At 12.20.0 ·00002 gram of pilocarpine in $\frac{1}{2}$ c.c. of normal saline was given.

12.21.0 Frequency 90 per minute. Systoles apparently stronger than in normal rhythm.

12.22.5 Frequency 104 per minute. Systoles stronger than in normal rhythm.

12.23.15 Frequency 106 per minute. Beats very strong.

12.25.0 Frequency 120 per minute. Beats very strong.

12.27.0 Frequency 130 per minute. Beats very strong.

12.28.0 Frequency 126 per minute. Beats very strong.

12.38.0 Frequency 120 per minute. Beats very strong.

12.39.0 Frequency 120 per minute. Beats very strong.

12.39.30 ·0002 gram of pilocarpine in $\frac{1}{2}$ c.c. of normal saline given.

12.46.0 During the last seven minutes the frequency was 126 per minute and the beats very strong.

12.47.0 ·0004 gram of pilocarpine in 1 c.c. of normal saline given.

12.48.0 Frequency 102 per minute. Beats very strong.

12.50.0 Frequency 92 per minute. Beats very strong.

12.55.0 During last five minutes rhythm was unchanged. ·0004 gram of pilocarpine in 1 c.c. of normal saline given.

12.59.0 Frequency during last four minutes 88 per minute. Beats very strong.

1.5.0 Rhythm unchanged during last six minutes. ·0004 gram of pilocarpine in 1 c.c. of normal saline given.

1.12.0 During the last seven minutes the frequency was 70 per minute and beats very strong.

1.14.0 $\frac{1}{2}$ c.c. containing .0004 gram of pilocarpine given.

1.20.0 Rhythm unchanged. 1 c.c. containing .004 gram of pilocarpine given.

1.29.0 Rhythm during last nine minutes 60 per minute.

1.30.0 1 c.c. containing .006 gram of pilocarpine given.

2.0.0 Rhythm 52 per minute. No change in its characteristics.

2.23.0 Rhythm 51 per minute and unchanged.

2.25.0 *Temperature of incubator raised to 42° C.*

2.30.0 Frequency during last four minutes 132 per minute. No change in the characteristics of the rhythm.

2.32.0 1 c.c. containing .02 gram of pilocarpine given.

2.34.0 Frequency 120 per minute. Beats fluttering and weak.

2.44.0 Frequency had fallen to 50 per minute. Beats regular and good.

2.45.0 1 c.c. containing .06 gram of pilocarpine given.

2.46.0 Frequency 42 per minute. Systoles weak.

2.52.0 Heart in diastolic expansion. It gave about 70 twitches during the minute.

2.55.0 Whole heart expanded, with both auricles and ventricles giving twitches but not synchronously.

2.56.0 Heart stopped in diastole, responds by one or two auricular twitches to mechanical stimulation of the auricles. I have failed to antagonise its action by potassium chloride.

Nov. 21st, 1892. Embryo aged 60 hours. Temperature throughout the experiment 38° C. Normal frequency 100 per minute.

3.34.0 $\frac{1}{2}$ c.c. of saline solution containing .005 gram of pilocarpine given.

3.35.0 Frequency 84 per minute. Beats very strong.

3.36.0 Frequency 94 per minute. Beats very strong.

3.43.0 Gradual decrease of rhythm during last seven minutes, the beats getting weaker and weaker until heart stops in *diastole*. Potassium chloride did not restore the rhythm.

Vulpian¹ injected extract of jaborandi under the skin covering the tibia of frogs and found that the heart-beats became irregular; the auricles were engorged with blood and did not contract completely at each systole, while the systoles of the ventricles were more complete. He concludes that jaborandi acts more rapidly on the auricles than on the ventricles. After fifteen minutes' action the whole heart stopped in full diastole.

He also stated that jaborandi does not slow the heart if urari had

¹ Vulpian. *Le Progrès Medical*, 1875. *Lond. Med. Record*, 1875, p. 398.

previously been given and concluded that its slowing was due to stimulation of the peripheral ends of the vagi.

Langley¹ finds that the ventricle is always the first to cease beating, but sometimes the ventricular rhythm may be stronger than the auricular rhythm. I find in the embryonic heart that the ventricles always stop first, and I have not obtained a reversed rhythm as in the action of certain other drugs where the ventricular rhythm has become dominant.

Langley found that jaborandi does slow the heart after urari poisoning and concluded that it acts upon something other than the inhibitory fibres going to the heart. He suggested that jaborandi acts on the neuromuscular mechanism of the cardiac tissue.

Pilocarpine evidently acts directly upon the embryonic myoplasm, apparently primarily stimulating it and secondarily depressing it. I have failed to antagonise pilocarpine by atropin on the embryonic heart. This may be due either to the large doses of pilocarpine used or to some difference between the embryonic and adult heart. If pilocarpine acts partially on the nervous elements of the frog's heart, this would account for the abnormal behaviour of atropin and pilocarpine on the embryonic heart.

Heat as in the case of many other drugs removes the effect of the pilocarpine. Further larger doses are required to produce the depression.

I have also failed to antagonise pilocarpine and potassium chloride in their action on the embryonic heart. I may here state that there are apparently different kinds of tonic and atonic standstill, for although the standstill induced by pilocarpine, morphine &c., is typically atonic, and apparently similar to the atonic standstill produced by nicotine and veratrine, yet potassium chloride will restore the rhythm in the latter case and not in the former case. Thus the chemical side of antagonism subdivides the wide group of substances which induce atonic standstill.

There is one general characteristic of atonic standstill if it be not carried to too great a limit, that whether it be produced by the action of chemical stimuli or simply by fall of temperature, it is removed by heating. The converse of this is not true, since although cooling will remove the tonic standstill produced by overheating the embryonic myoplasm, yet cooling will not remove the tonic standstill produced on the embryonic myoplasm by drugs like caffeine and digitalin.

General Remarks.

The presence of rhythmic activity in the embryonic heart previous

¹ Langley. *Journ. Anat. and Phys.* Vol. x. (1876), p. 186.

to the development of a functional nervous system, puts almost beyond doubt the view that the rhythmic activity of the heart is a function of its contractile tissue independent of nervous agencies. What then is the function of the intrinsic cardiac nervous mechanism? Besides that of conveying and distributing impulses from without (via vagus and sympathetic) is it not possible judging from the evidence deduced in the section on atropin and muscarin that the terminal fibrils of the cardiac nerves may react to chemical stimulation and thus influence the rhythm of the heart? Is it not also possible that even if playing a secondary part in the normal sequence of the cardiac cycle, they may when stimulated by the selective action of certain drugs influence the rhythm of the heart? This question will I believe only be decided by further experiments, and part of the object of this paper is to reopen the line of investigation of the functions of the intrinsic cardiac nervous mechanism.

Summary of Chief Results.

The chief facts and their bearing have been dealt with section by section. The following summary is given to shew the general import of the paper:

1. That the embryonic heart of the chick will if kept with proper precautions beat with a rhythm constant for each individual embryo, and that as it is easily worked with previous to the development of a functional nervous system it affords a means of differentiating the functions of cardiac muscle from the nerves which supply it.
2. That the embryonic heart reacts to all classes of stimuli (chemical, thermal, and electrical) which influence the adult heart.
3. That the embryonic heart is delicately sensitive to thermal stimuli. That each heart has a certain temperature at which its rhythmic action is most marked. That temperatures above and below this point depress the cardiac rhythm. That the heart when overheated passes into a condition of idiomuscular contraction, but the final heat stoppage is in diastole. That small variations of temperature over long periods of time do not influence the cardiac rhythm.
4. That the embryonic myoplasm conducts mechanical stimuli from the ventricular to the auricular end of the heart.
5. That caffeine slightly increases the frequency of the embryonic heart besides increasing the energy of the systoles. That in larger doses it causes tonic contraction of the embryonic myoplasm, culminating in systolic stoppage. That these results may explain certain phenomena shewn by caffeine on the adult heart.

6. That xanthine causes a primary depression of the embryonic heart, followed by a secondary increase both in force and frequency. That the condition of idiomuscular contraction was not obtained even with large doses of xanthine.

7. That theobromine causes a primary increase of frequency of the embryonic heart. That it also causes the systoles to become stronger, but that in large doses it has little or no depressant effect. Though the tonic contraction of the heart was more marked than when under the influence of xanthine, yet it was but slight when compared with that produced by caffeine.

8. That the introduction of methyl groups into the xanthine molecule modifies the action of that molecule on the embryonic heart, and that the greater the number of methyl groups introduced, the greater is the tonic contraction of the embryonic heart.

9. The embryonic heart under the influence of digitalin behaves like the adult heart.

10. That the action of strophanthin on the embryonic heart is apparently identical to its action on the adult heart. That these results strongly confirm the views which Fraser arrived at from observations on the frog's heart.

11. That hydrocyanic acid has probably a complex action on the embryonic heart, owing partly to the cyanhaemoglobin formed and in part to its direct action as an acid. That the primary action causes reversal of the normal rhythm. That the secondary action causes diastolic stoppage.

12. That amyl nitrite also probably has a mixed action due to the methaemoglobin formed, and possibly to its action direct on the cardiac myoplasm. That in its primary phase of action it also reverses the cardiac rhythm.

13. That morphine similarly depresses the cardiac rhythm and reverses it.

14. That there is possibly some connection between the reversal of cardiac rhythm and the depression of processes of oxidization in the contractile substance of the heart.

15. That potassium chloride stops the embryonic heart in diastole.

16. That nicotine causes a primary acceleration followed by depression and diastolic stoppage of the embryonic heart. That nicotine is antagonized by potassium chloride. There is a quantitative relationship between the amount of nicotine that is antagonized by potassium chloride.

17. That the antagonism of nicotine and potassium chloride on the embryonic heart is probably chemical in nature, and that the tone of heart probably plays a secondary rôle.

18. That veratrine in small doses causes an acceleration of the embryonic heart's action, in larger doses it depresses its action by lengthening out its systoles. It is antagonized by potassium chloride.

19. That there is a certain temperature at which the action of a drug is most potent on the embryonic heart. That temperatures above and below this point modify the action of the drugs. That the temperature of maximum action is higher in the chick-embryo than in the frog's heart.

20. That the embryonic heart has a normal tone, and that tone can exist independent of nervous action. That the tone of the embryonic heart is modified by the direct action of drugs upon the embryonic myoplasm. That there may be some connection between the oxidizing or reducing action of a drug and its influence on the tone of the embryonic heart.

21. That the atonic condition can usually be removed by the application of heat. That the converse is not so, except in the case of idiomuscular contraction produced by overheating.

22. That the antagonism between the action of calcium salts and potassium salts on the heart is remarkable, since the two salts are antagonistic in blood coagulation and in the molecular changes of rigor mortis. That further investigation should be made along this line.

23. That the action of chloroform and ether on the embryonic heart is distinct. That ether primarily is a stimulant and chloroform a depressant. That chloroform causes extreme dilatation of the embryonic heart. That the action of chloroform is partially antagonized by ammonia.

24. That ammonia is a powerful stimulant of the embryonic heart. In large doses it is fatal in diastole.

25. That tetraethylammonium hydroxide is also fatal in diastole. That the introduction of ethyl groups into the ammonium molecule does not modify the action of its hydroxides.

26. That barium chloride is a marked depressant to the embryonic heart.

27. That muscarin nitrate has no specific action on the embryonic heart.

28. That atropin has only a comparatively small depressant action on the embryonic heart.

29. That the action of muscarin and atropin is not constant on the hearts of different invertebrates.

30. That it is not improbable that the nerve endings in the adult heart may be influenced by atropin and muscarin. That the subject requires further investigation along the lines indicated.

31. That strychnine causes a temporary acceleration of the embryonic heart, and in larger doses depression until it stops the heart in diastole. There is no spasm at any phase of its action.

32. That pilocarpine acts directly on the embryonic myoplasm, primarily stimulating it and secondarily depressing it.

In conclusion it is my pleasant duty to record my warmest thanks to Prof. Halliburton for the kindly advice and criticism he has given during my work, as well as for his readiness to witness my experiments. To Prof. Klein I am greatly indebted for much advice. I hope in a subsequent paper to continue this research.

DESCRIPTION OF PLATE.

Fig. I. is a diagram of the observing incubator.

jj. = water jacket. *a.s.* = air space. *i.jj.* = pipe for filling water jacket. *t.* = tap for emptying water jacket. *e.s.* = copper strips and binding screws which suspend the egg in the air chamber. *w.l.* = wooden frame which carries the suspensory apparatus and prevents rapid conduction of heat to the egg. *e.* = egg. *w.v.* = small vessel of water to keep air chamber moist. *gl.s.* = sheet of glass closing in the top of the chamber.

Fig. II. Diagram of effect of temperature on the embryonic heart, the ordinates represent the number of beats per minute, the abscissæ the degrees of temperature. The curve should be read from left to right.

Fig. III. Diagram of the effect of caffeine on the embryonic heart. The abscissæ represent time, the ordinates beats per minute. The larger circles represent the introduction of the drug. The temperature remains constant and is therefore not represented.

Fig. IV. Diagram of the action of theobromine on the embryonic heart. The abscissæ represent time, the ordinates beats per minute.

Fig. V. Similar diagram of the action of xanthine on the embryonic heart.

Fig. VI. Similar diagram shewing the action of nicotine on the embryonic heart. The dotted lines in this diagram represent the period of time during which the heart was stopped. The antagonism existing between nicotine and potassium chloride is here illustrated.

Fig. VII. Similar diagram illustrating the antagonism between veratrine and potassium chloride on the embryonic heart.

Fig. VIII. Diagram illustrating the reversal of rhythm which occurs in the final phase of amyl nitrite poisoning of the embryonic heart.

(a) = comparatively rapid beating. (b) = phase when embryonic heart shews Luciani's beats. (c) = phase of reversed rhythm represented by beats below the base line. (d) = divisions shewing seconds. This diagram does not represent the force of the beats.

Feb. 9, 1893.

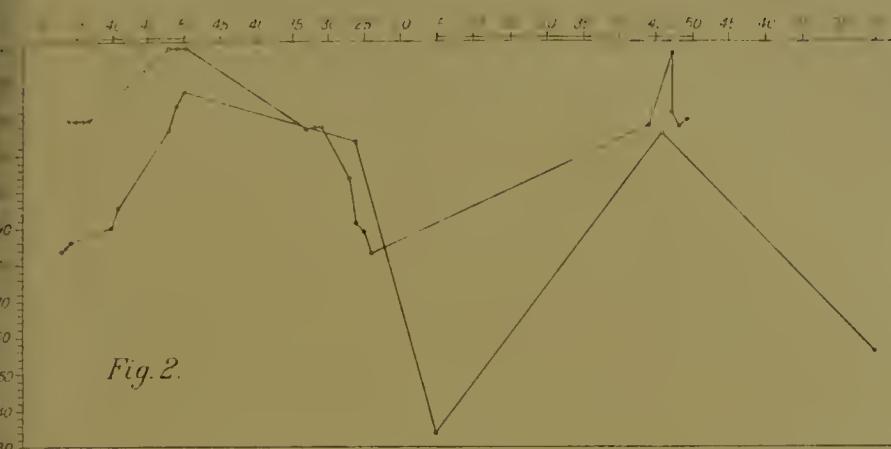


Fig. 2.

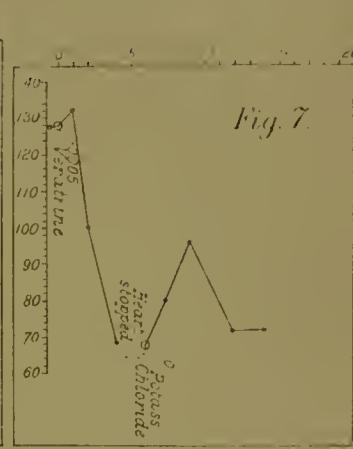


Fig. 7.

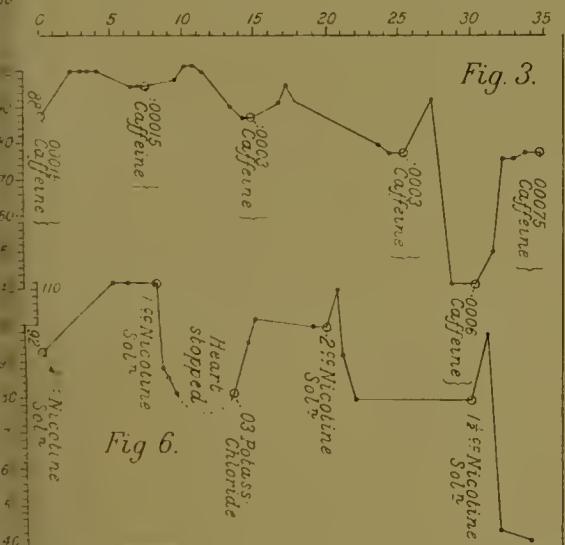


Fig. 3.

Fig. 6.

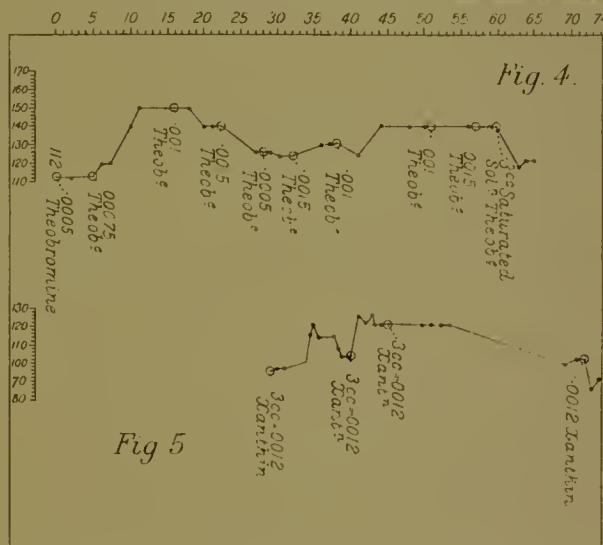
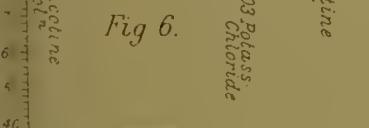


Fig. 4.

Fig. 5

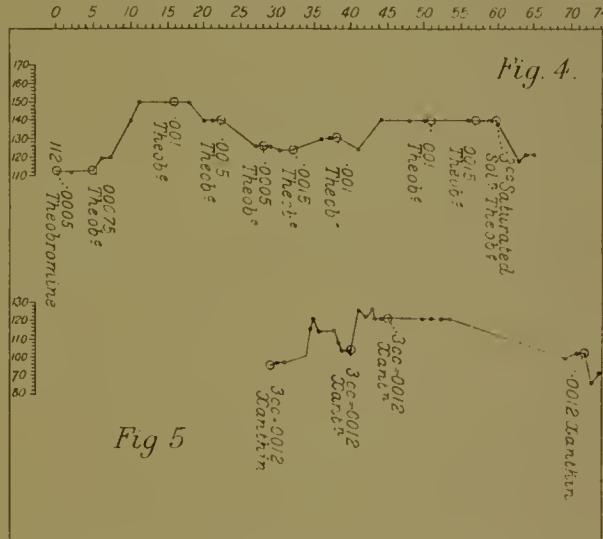


Fig. 5

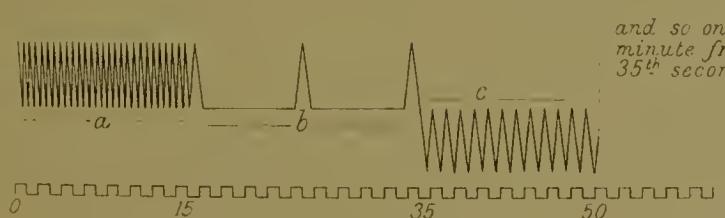


Fig. 8.

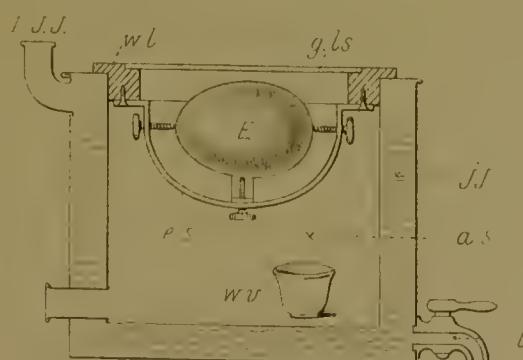


Fig. 1.

